



INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification ⁶ : C12N 15/13, 15/31, 15/62, 15/82, C07K 16/18, 16/28, C12N 5/10, A01H 5/00, C12N 1/21, C12P 21/08, C12N 5/12, A01N 63/02	A1	(11) International Publication Number: WO 96/00783 (43) International Publication Date: 11 January 1996 (11.01.96)
(21) International Application Number: PCT/IB95/00497 (22) International Filing Date: 20 June 1995 (20.06.95) (30) Priority Data: 08/267,641 28 June 1994 (28.06.94) US (60) Parent Application or Grant (63) Related by Continuation US 08/267,641 (CIP) Filed on 28 June 1994 (28.06.94) (71) Applicant (for all designated States except US): CIBA-GEIGY AG [CH/CH]; Klybeckstrasse 141, CH-4002 Basle (CH). (72) Inventors; and (75) Inventors/Applicants (for US only): CAROZZI, Nadine, Bar- bara [US/US]; 6900 Laurdane Road, Raleigh, NC 27612 (US). KOZIEL, Michael, Gene [US/US]; 509 Carolyn Court, Cary, NC 27511 (US). (74) Common Representative: CIBA-GEIGY AG; Patentabteilung, Klybeckstrasse 141, CH-4002 Basle (CH).	(81) Designated States: AM, AU, BB, BG, BR, BY, CA, CN, CZ, EE, FI, GE, HU, IS, JP, KG, KP, KR, KZ, LK, LR, LT, LV, MD, MG, MN, MX, NO, NZ, PL, RO, RU, SG, SI, SK, TJ, TM, TT, UA, US, UZ, VN, European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG), ARIPO patent (KE, MW, SD, SZ, UG). Published <i>With international search report.</i>	
(54) Title: ANTIBODIES WHICH BIND TO INSECT GUT PROTEINS AND THEIR USE		
(57) Abstract Antibodies, monoclonal antibodies or fragments thereof which bind to brush border membrane vesicles of insect gut and the gene or genes which encode these proteins are provided. The monoclonal antibodies bind the gut of a target insect but do not bind to mammalian brush border membranes or to plant microsomes. The antibodies and the genes encoding them find use in constructing hybrid toxins for control of insect pest.		

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AT	Austria	GB	United Kingdom	MR	Mauritania
AU	Australia	GE	Georgia	MW	Malawi
BB	Barbados	GN	Guinea	NE	Niger
BE	Belgium	GR	Greece	NL	Netherlands
BF	Burkina Faso	HU	Hungary	NO	Norway
BG	Bulgaria	IE	Ireland	NZ	New Zealand
BJ	Benin	IT	Italy	PL	Poland
BR	Brazil	JP	Japan	PT	Portugal
BY	Belarus	KE	Kenya	RO	Romania
CA	Canada	KG	Kyrgyzstan	RU	Russian Federation
CF	Central African Republic	KP	Democratic People's Republic of Korea	SD	Sudan
CG	Congo	KR	Republic of Korea	SE	Sweden
CH	Switzerland	KZ	Kazakhstan	SI	Slovenia
CI	Côte d'Ivoire	LI	Liechtenstein	SK	Slovakia
CM	Cameroon	LK	Sri Lanka	SN	Senegal
CN	China	LU	Luxembourg	TD	Chad
CS	Czechoslovakia	LV	Larvia	TG	Togo
CZ	Czech Republic	MC	Monaco	TJ	Tajikistan
DE	Germany	MD	Republic of Moldova	TT	Trinidad and Tobago
DK	Denmark	MG	Madagascar	UA	Ukraine
ES	Spain	ML	Mali	US	United States of America
FI	Finland	MN	Mongolia	UZ	Uzbekistan
FR	France			VN	Viet Nam
GA	Gabon				

ANTIBODIES WHICH BIND TO INSECT GUT PROTEINS AND THEIR USE

The invention is drawn to antibodies that bind to insect gut proteins and their use, particularly their use in creating new hybrid toxin molecules. The invention further relates to microorganisms, plant cells and plants producing the said antibodies and hybrid toxins, respectively. The invention also encompasses insecticidal compositions and the use thereof in protecting plants against insect pests.

Control of various pests through the use of biological molecules has been possible in only a limited number of cases. The best known examples of biological molecules with pesticidal uses are the δ -endotoxins from *Bacillus thuringiensis* (Bt). Various strains of Bt are known which produce insecticidal proteins, the δ -endotoxins, during sporulation. Some of these δ -endotoxins have useful insecticidal activities against different insect pests. However, use of the δ -endotoxins is limited because they are active against only a very few of the many insect pests.

The limited specificity of the Bt endotoxins is dependent, at least in part, on both the activation of the toxin in the insect gut (Haider, M. Z. *et al.*, 1986, Eur. J. Biochem. 156:531-540) and its ability to bind to specific receptors present on the insect's midgut epithelial cells (Hofmann, C. P. *et al.*, 1988, PNAS 85:7844-7848). Among the factors which prevent activity of a particular Bt δ -endotoxin against a specific insect is the lack of appropriate receptors in the insect gut or lack of affinity of the δ -endotoxin for the receptors which may be present, thus resulting in no binding of the δ -endotoxin to the brush border membranes. Therefore, the ability to control a specific insect pest using Bt δ -endotoxins at present depends on the ability to find an appropriate δ -endotoxin with the desired range of activity. In many cases, no such δ -endotoxin is known, and it is not certain that one even exists. For example, thousands of Bt strains have been screened for activity against western corn rootworm (WCRW), a major pest of maize. However, to date there are no reports of strains of Bt which produce a δ -endotoxin that is highly effective against WCRW.

Individual δ -endotoxins typically have a very narrow spectrum of activity, each being active against only one or a few insect pests. Moreover, the δ -endotoxins have been shown to be active against only a few members of but a small number of Orders of insects. The ability to produce additional proteins with unique pesticidal activities creates more options for

the control of agricultural pests, particularly insects, using biological molecules with a high level of safety for non-target organisms. Thus, there is a need for binding proteins which can be designed to target a particular insect pest.

Hence, the present invention is drawn to antibodies, but especially to monoclonal antibodies or fragments thereof which bind to brush border membrane vesicles of the insect gut and to the gene or genes which encode these proteins. The antibodies according to the invention bind to proteins in the gut of a target insect especially to a target insect selected from the orders Coleoptera, Diptera, Hymenoptera, Lepidoptera, Mallophaga, Homoptera, Hemiptera, Orthoptera, Thysanoptera, Dermaptera, Isoptera, Anoplura, Siphonaptera, and Trichoptera but do not bind to mammalian brush border membranes or to plant microsomes. In particular the invention relates to a monoclonal antibody or fragment which binds to the gut of the western corn root worm. In a preferred embodiment, the invention relates to a monoclonal antibody which is selected from the group consisting of 2B5, 3B1, 10B6, 17F6, 14G1 and 16E4.

Also comprised by the invention are hybridoma cell lines producing the monoclonal antibodies according to the invention, but in particular hybridoma cell lines which have been deposited under accession number ATCC HB 11616, HB 11617, HB 11618, HB 11619 and HB 11620.

It is a further object of the invention to provide a DNA sequence which encodes a monoclonal antibody or the binding site of said monoclonal antibody according to the invention. In particular the invention relates to a DNA sequence encoding a monoclonal antibody or the binding fragment thereof wherein said DNA sequence is selected from SEQ. ID Nos 1, 3, 5, 7, 9, 11, 13, 15, 17, 44 or 46. Also included is a DNA sequence wherein said DNA sequence is operably linked to a toxin moiety, especially wherein said toxin moiety is selected from *Bacillus* toxins, *Pseudomonas* exotoxin, phytolaccin, gelonin, ribonucleases or ribosome inactivating proteins.

The antibodies and the genes encoding them may find use in constructing hybrid-toxins for control of insect pests. Hence, an additional aspect of the invention is a hybrid toxin molecule comprising the monoclonal antibody or monoclonal antibody binding fragment according to the invention operably linked with a toxin moiety. In a preferred embodiment of the invention the said toxin moiety is selected from *Bacillus* toxins such as, for example, *Bacillus* endotoxins but especially from toxins selected from the group consisting of *Bt*

endotoxin, vegetative insecticidal proteins, *Pseudomonas* exotoxin, phytolaccin, gelonin, ribonucleases and ribosome inactivating proteins.

The invention thus further relates to a hybrid toxin comprising a monoclonal antibody or binding region thereof which binds to the gut of a target insect but especially to insect brush border membranes but does not bind to mammalian brush border membranes or to plant microsomes operably linked to a toxin moiety wherein the toxin moiety is selected from *Bacillus* toxins such as, for example, *Bacillus* endotoxins but especially from toxins selected from the group consisting of *Bt* endotoxin, vegetative insecticidal proteins, *Pseudomonas* exotoxin, phytolaccin, gelonin, ribonucleases and ribosome inactivating proteins.

The invention further encompasses a DNA sequence which encodes a hybrid toxin according to the invention comprising a monoclonal antibody or binding region thereof which binds to the gut of a target insect but does not bind to mammalian brush border membranes or to plant microsomes operably linked to a toxin moiety.

The invention further encompasses a microbial host which is transformed with a suitable vector in which a DNA molecule encoding the hybrid toxin according to the invention is inserted. In particular the microorganism host include bacteria, algae and fungi.

The present invention thus further comprises a recombinant microorganism, but especially a microorganism selected from the group consisting of bacteria such as *Bacillus*, *Caulobacter*, *Agmenellum*, *Pseudomonas*, *Erwinia*, *Serratia*, *Klebsiella*, *Xanthomonas*, *Streptomyces*, *Rhizobium*, *Rhodopseudomonas*, *Methylius*, *Agrobacterium*, *Acetobacter*, *Lactobacillus*, *Arthrobacter*, *Azotobacter*, *Leuconostoc*, and *Alcaligenes*; fungi, particularly yeast, such as *Saccharomyces*, *Cryptococcus*, *Kluyveromyces*, *Sporobolomyces*, *Rhodotorula*, and *Aureobasidium* and viruses such as the nuclear polyhedrosis virus *Autographica californica*, transformed with at least one of the DNA sequences according to the invention.

In particular, the invention relates to a recombinant microorganism comprising at least one DNA molecule encoding a hybrid toxin comprising an antibody or fragment thereof which binds to the gut of a target insect but does not bind to mammalian brush border membranes or to plant microsomes operably linked to a toxin moiety wherein the said DNA encoding a monoclonal antibody comprises a sequence selected from the group consisting of SEQ. ID Nos 1, 3, 5, 7, 9, 11, 13, 15, 17, 44 or 46.

The invention further encompasses an entomocidal composition comprising any of the above mentioned recombinant microorganisms according to the invention in an insecticidally-effective amount together with a suitable carrier.

In particular, the invention relates to an entomocidal composition comprising an isolated hybrid toxin molecule according to the invention in an insecticidally-effective amount together with a suitable carrier.

The invention further relates to a DNA sequence consisting of a first expression cassette comprising a DNA sequence which encodes a promoter capable of directing expression in a plant operably linked to a first DNA sequence which encodes a light chain of a monoclonal antibody or antibody binding domain wherein said monoclonal antibody binds the gut of a target insect. In a further embodiment said cassette further comprises a second DNA sequence which encodes a toxin moiety operably linked to the said first DNA sequence which encodes the light chain of the monoclonal antibody or antibody binding domain. Especially preferred is an expression cassette wherein the second DNA sequence encodes a toxin moiety selected from the group consisting of *Bacillus* toxins, *Pseudomonas* exotoxin, phytolectin, gelonin, ribonucleases and ribosome inactivating proteins.

The invention further encompasses a DNA sequence comprising a second expression cassette comprising a DNA sequence encoding a promoter capable of directing expression in a plant operably linked to a first DNA sequence which encodes a heavy chain of a monoclonal antibody or antibody binding domain wherein said monoclonal antibody binds the gut of a target insect. In a further embodiment said cassette further comprises a second DNA sequence which encodes a toxin moiety operably linked to the said first DNA sequence encoding the heavy chain of the monoclonal antibody or antibody binding domain. Especially preferred is an expression cassette wherein the second DNA sequence encodes a toxin moiety selected from the group consisting of *Bacillus* toxins, *Pseudomonas* exotoxin, phytolectin, gelonin, ribonucleases and ribosome inactivating proteins.

The DNA sequence according to the invention may be present either in an isolated and essentially purified form or as part of a plant genome.

The invention further relates to plant cells or plants including the progeny thereof, but especially to maize plants, which plant cells or plants are transformed with a DNA sequence according to the invention, but especially with a plant expression cassette according to the invention. The invention further embodies transgenic plant cells or plants including the progeny thereof, but especially maize plants, which express a hybrid toxin according to the

invention, especially in an amount sufficient to render the plant cell and plant, respectively, tolerant or resistant to insect pests. The plants according to the invention are preferably hybrid plants.

Also comprised within the scope of the invention are plant propagules but especially plant seeds that are treated with a protectant coating.

The invention further relates to a process for the preparation of a hybridoma cell line which produces an antibody or monoclonal antibody according to the invention comprising

- a) utilizing insect guts, particularly insect brush border membranes, as the antigen;
- b) immunising a donor animal with said antigen;
- c) isolating an immunocompetent B cell from the immunised donor animal;
- d) fusing said immunocompetent B cell with a tumour cell line which is capable of continuous cell division;
- e) isolating the resulting fusion product, cultivating it in a suitable culture medium and subsequently cloning positive hybrid cells; and
- f) screening the cloned hybrid cells for the production of monoclonal antibodies, and selecting those which show the required properties.

The invention further relates to a method of producing an antibody, but especially a monoclonal antibody or fragments thereof which bind to brush border membrane vesicles of insect gut comprising growing the hybridoma cell lines producing the said antibodies in a suitable cultivation medium *in vivo* or *in vitro* and isolating the resulting antibodies.

The invention further relates to a method of producing hybrid toxins comprising the monoclonal antibody or monoclonal antibody binding fragment according to the invention operably linked with a toxin moiety which bind to brush border membrane vesicles of the insect gut comprising cloning the variable region of the said antibodies and linking said region to the toxin moiety.

Further comprised by the invention is a process of producing a DNA sequence encoding a monoclonal antibody according to the invention comprising cloning the respective antibody genes from hybridoma cells using primers to conserved DNA sequences within the constant regions and the framework regions of the variable regions.

The invention further encompasses a method of producing a transgenic plant cell and plant, respectively comprising transforming the said plant cell or plant with one or more of the aforementioned DNA sequences encoding a hybrid toxin according to the invention

comprising a monoclonal antibody or binding region thereof which binds to the gut of a target insect but does not bind to mammalian brush border membranes or to plant microsomes operably linked to a toxin moiety.

Antibodies and monoclonal antibodies, including fragments thereof which are capable of binding with the specificity of the antibody or monoclonal antibody, to proteins found in the insect gut are provided. Such antibodies bind to insect gut cells but do not bind to mammalian brush border membrane vesicles (BBMVs), nor to plant microsomes.

The antibodies of the invention include polyclonal and monoclonal antibodies as well as fragments thereof which retain their ability to bind to proteins found in the insect gut. An antibody, monoclonal antibody, or fragment thereof is said to be capable of binding a molecule if it is capable of specifically reacting with the molecule to thereby bind the molecule to the antibody, monoclonal antibody, or fragment thereof. The term "antibody" (Ab) or "monoclonal antibody" (Mab) is meant to include intact molecules as well as fragments or binding regions or domains thereof (such as, for example, Fab and F(ab)₂ fragments) which are capable of binding hapten. Such fragments are typically produced by proteolytic cleavage, such as papain or pepsin. Alternatively, hapten-binding fragments can be produced through the application of recombinant DNA technology or through synthetic chemistry.

Methods for the preparation of the antibodies of the present invention are generally known in the art. For example, see Antibodies, A Laboratory Manual, Ed Harlow and David Lane (eds.) Cold Spring Harbor Laboratory, NY (1988), as well as the references cited therein. Standard reference works setting forth the general principles of immunology include: Klein, J. Immunology: The Science of Cell-Noncell Discrimination, John Wiley & Sons, NY (1982); Dennett, R., *et al.* Monoclonal Antibodies, Mybridoma: A New Dimension in Biological Analyses, Plenum Press, NY (1980); and Campbell, A. "Monoclonal Antibody Technology," In Laboratory Techniques in Biochemistry and Molecular Biology, Vol. 13, Burdon *et al.* (eds.), Elsevier, Amsterdam (1984). See also, US Patent Nos: 4,609,893; 4,713,325; 4,714,681; 4,716,111; 4,716,117; and 4,720,459.

The antibody and monoclonal antibodies of the present invention can be prepared by utilizing insect guts, particularly insect brush border membranes, as the antigen. Such insect gut membranes can be prepared by methods known in the art. Generally, brush border membranes can be isolated from insect larvae by dissection of guts and

homogenization followed by calcium chloride precipitation of membranes. See, for example, Wolfersberger (1986) *Comp. Biochem. Physiol.* 86A:301-308.

It is recognized that following the methods described herein, antibodies specific for a particular target insect can be prepared. By target insect is meant an insect in which the antibodies of the present invention will bind to protein or proteins present in the gut. That is, antibodies can be prepared that are capable of binding proteins present in the gut of only the target insect.

The target insect encompasses any insect including insects selected from the orders Coleoptera, Diptera, Hymenoptera, Lepidoptera, Mallophaga, Homoptera, Hemiptera, Orthoptera, Thysanoptera, Dermaptera, Isoptera, Anoplura, Siphonaptera, Trichoptera, etc. Thus, any insect pest can be selected and antibodies made which are specific to that insect. Of particular interest are insect pests for which there is no Bt protein which is capable of binding and killing, such as western corn rootworm.

The antibody and monoclonal antibody producing cell lines of the invention are a subset of all monoclonal antibodies produced when insect brush border membrane vesicles (BBMVs) are used as antigen for the production of MAb lines. The binding characteristics of the desired monoclonal antibody producing cell lines are determined by differentially screening all of the various monoclonal antibodies raised against the BBMVs of the target insect.

The differential screen of the present invention identifies the antibody lines which also bind mammalian BBMVs and/or microsomes of plants. MAb cell lines which bind to mammalian BBMVs or to plant microsomes are discarded. A differential screen can also identify Mab cell lines which bind BBMV of insects in species other than the target insect. Thus, the antibodies of the invention are those which demonstrate highly selective binding for only target insects, especially for the gut of a target insect.

The subset of MAb lines which possess the desired binding specificity can be used as a source of messenger RNA for cloning of the cDNA for the particular monoclonal antibody. Antibody genes can be cloned from hybridoma cells using primers to conserved DNA sequences within the constant regions and the framework regions of the variable regions. This can be followed by amplification of the DNA for cloning using the polymerase chain reaction (PCR). A database of mouse heavy chain and light chain sequences compiled by Kabat *et al.* has been successfully used to generate both isotype specific and degenerate primers for cloning antibody genes (Kabat, E. A. *et al.*, 1987, US Dept Health and Human

Services, US Government Printing Offices and Jones, S. T. and Bendig, M., 1991, Bio/technology 9:88-89). Additionally, there is a wealth of knowledge concerning the cloning of smaller fragments of antibodies which possess the binding properties of the original antibody.

The cloned DNA can then be sequenced by methods known in the art. See, for example, Sambrook *et al.*, Molecular Cloning: A Laboratory Manual, 2nd. Edition, Cold Spring Harbor Laboratory Press, NY (1989) vol. 1-3, and the references cited therein. From the nucleic acid sequence, the protein sequence of the binding region from the selected MAb can be deduced.

The antibodies and monoclonal antibodies of the invention find use in the production of hybrid toxin molecules. By "hybrid toxin molecules" or "hybrid toxins" is intended, fusion proteins or immunotoxins, which comprise a monoclonal antibody or antibody fragment operably linked to a toxin moiety and which is capable of binding to the gut of an insect. That is, when linked, the monoclonal antibody or antibody fragment retains its binding properties and the toxin moiety retains its cytotoxic properties.

A number of cytotoxic proteins can be utilized as the toxin moiety. These include but are not limited to *Bacillus* toxins, including endotoxins and vegetative insecticidal proteins. See for example US application serial no. 08/037,057, filed March 25, 1993 and WO 93/07278, herein incorporated by reference. Other toxins include catalytic ribosome inactivators such as gelonin, *Pseudomonas* exotoxin A or phytolaccin, (the structure of *Pseudomonas* exotoxin has been well characterized in Chaudhary *et al.*, (1990) J. Biol. Chem. 265:16303-16310); cell metabolism disrupters, such as ribonucleases, (see, for example, Mariani *et al.* (1990) Nature 347:737-741); Barnase toxin (or PE-Bar), a chimeric toxin derived from *Pseudomonas* exotoxin A and a ribonuclease, (see, Prior *et al.* (1991) Cell 64:1017-1023); hydrophilic peptides that create pores in membranes (see, Frohlich and Wells (1991) Int. J. Peptide Protein Res. 37:2-6); etc.

The hybrid toxin molecules of the present invention therefore contain a region which allows binding of the molecule to insect guts (antibody region) as well as a toxic region to effect killing of the targeted cell and ultimately the targeted insect. By utilizing the monoclonal antibodies or fragments thereof in the hybrid toxins, the hybrid toxins bind to the gut of a target insect and thereby exert a toxic effect on only that insect. The binding characteristics of such hybrid toxins are derived from the MAb binding region while the toxic effect of such hybrid toxins is derived from the toxic moiety used.

Methods for linking the antibody or antibody fragments to the toxins are known in the art. Such methods include linkers used in single chain antibody immunotoxins (Chaudhary *et al.* (1989) *Nature* 339:394-397; Chaudhary *et al.* (1990) *PNAS* 87:9491-9494; Batra *et al.* 1991, *Mol. and Cellular Biol.* 11:2200-2205; Brinkmann, *et al.* (1991), *PNAS* 88:8616-8620; Brinkmann *et al.* (1992) *PNAS* 89:3075-3079; Whitlow *et al.* (1993) *Protein Engineering* 6:989-995). One particularly useful linker is based on the human IgA1 hinge region as reported by Hallewell *et al.* (1989) *J. Biol. Chem.* 264: 5260-5268 and described in SEQ ID NO:43.

The activity of the hybrid toxin molecules may depend on several factors which can be optimized. The activity can be assayed using protein produced by transiently expressing maize protoplasts. In this manner, maize protoplasts expressing the hybrid toxins can be incorporated into insect diet for activity assays. For general insect assays, see Marrone (1985) *J. Econ. Entomolo.* 78:290-293, MacIntosh *et al.* (1990) *J. of Invertebrate Pathology* 56:258-266 and the references cited therein.

Thus, hybrid toxin constructs can be tested for insecticidal activity against the target pest of interest. Those constructs exhibiting activity can be further developed for agricultural use.

It is further recognized that various constructs of hybrid toxins can be generated. For example, the hybrid toxin could be encoded by two expression cassettes which respectively encode the light and heavy chains of the antibody molecule. This binary hybrid toxin can then be assembled *in vivo* using the normal processing machinery of the cell to create the antibody binding site. The toxin moiety of the hybrid can be operably linked to the N or C terminal of the light or heavy chain or alternatively could replace any or part of the constant regions of either chains. The toxin moiety could also be inserted within a constant region or between constant regions of the antibody chains. Such constructions can be made by standard molecular techniques. See, for example Sambrook *et al.*, *Molecular Cloning: A Laboratory Manual*, 2nd. Edition, Cold Spring Harbor Laboratory Press, NY (1989) vol. 1-3 and the references cited therein.

The hybrid toxins of the present invention, including binary toxins, can be produced in plants. In this manner, the antibody genes can be cloned and expressed in plants in such a manner that functional antibodies are assembled. See, for example, Hiatt *et al.* (1989) *Nature* 342:76-78 During *et al.* (1990) *J. Plant Molecular Biology* 15:281-293 and PCT Application WO 91/06320. Levels of bivalent antibody expression have been reported to be

as high as 1% of the soluble protein in tobacco. It is recognized that as well as antibody molecules, antibody fragments such as Fab and Fv fragments, can be utilized. The smaller Fab and Fv antigen-binding fragments (12 kDa-50 kDa) have been shown to retain full binding affinity. Single chain Fv fragments (scFv), in which Vh and Vl domains are linked by a hydrophilic and flexible peptide, have been used successfully to target enzymes and toxins to specific cells (Bird (1988) Science 423:423-426 and Huston (1988) PNAS 85:5879-5883). Single Vh domains (Dabs) and single complementary determining regions as small as 20 amino acids (aa) called minimal recognition units (mru) have also been used for antigen binding (Ward (1989) Nature 341:544-546 and Taub (1989) J. Biol. Chem 264:259-265 and Williams (1989) PNAS 86:5537-5541). The use of these antibody fragments provides the option of reducing the insect specific binding domain derived from a MAb to a very small size.

DNA fragments encoding antibodies, or regions of antibodies, which bind to the gut of insects are also encompassed by the present invention. In a preferred embodiment, these DNA fragments encode binding regions which are derived from monoclonal antibodies raised against BBMV of a desired target insect and screened to ensure they do not bind to mammalian BBMV or to plant microsomes. Such DNA fragments can be used in the construction of genes encoding novel hybrid toxin molecules which are discussed above.

DNA sequences encoding the toxin moiety of the hybrid toxins are known in the art. See, Lamb *et al.* (1985) Eur. J. Biochem. 148:275-170 (Ricin); Gray *et al.* (1984) PNAS 81:2645-2649 (*Pseudomonas* toxin DNA Sequence); Hindley and Berry (1988) Nuc. Acids Res. 16:4168 (*B.sphaericus* toxin gene); Bauman *et al.* (1988) J. Bacteriol. 170:2045-2050, Baumann *et al.* 1987) J. Bacteriol. 169:4061-4067, Berry and Hindley (1987) Nucleic Acids Res. 15:5891, Berry *et al.* (1989) Nucleic Acids Res. 17:7516 (*B. sphaericus*); WO 9309130-A (gelonin); EP 466222-A, U.S. Patent No. 5,128,460 (ribosome-activating protein); EP 412911-A (barnase); Heemstadt *et al.* (1987) Gene 57:37-46 (*cryIIIA*); Brizzard and Whiteley (1988) Nucleic Acids Res 16:2723-2724 (*cryIB*); and Geiser *et al.* (1986) Gene 48:109-118 (*cryIA(b)*). See also Porter *et al.* (1993) Microbiological Reviews 57:838-861; Hofte and Whiteley (1989) Microbiological Reviews 53:242-255; and WO 93/07278.

The hybrid toxin genes of the invention can be optimized for enhanced expression in plants. See, for example WO 93/07278; EPA 0359472; EPA 0385962; WO 91/16432; Perlak *et al.* (1991) Proc. Natl. Acad. Sci. USA 88:3324-3328; and Murray (1989) Nucleic Acids Research 17: 477-498. In this manner, the genes can be synthesized utilizing plant preferred codons. That is, the preferred codon for a particular host is the single codon which

most frequently encodes that amino acid in that host. The maize preferred codon, for example, for a particular amino acid may be derived from known gene sequences from maize. Maize codon usage for 28 genes from maize plants is found in Murray, (1989), *Nucleic Acids Research* 17:477-498, the disclosure of which is incorporated herein by reference. Synthetic genes could also be made based on the distribution of codons a particular host uses for a particular amino acid.

Following this approach, the nucleotide sequences can be optimized for expression in any plant. It is recognized that all or any part of the gene sequence may be optimized or synthetic. That is, synthetic, partially optimized, or native sequences may also be used.

Methods for the transformation of plant cells and regeneration of transformed plants are well known in the art. Generally, for the introduction of foreign DNA into plants Ti plasmid vectors have been utilized as well as direct DNA uptake, liposomes, electroporation, micro-injection, and the use of microprojectiles. Such methods have been published. See, for example, Guerche *et al.*, (1987) *Plant Science* 52:111-116; Neuhauser *et al.*, (1987) *Theor. Appl. Genet.* 75:30-36; Klein *et al.*, (1987) *Nature* 327: 70-73; Howell *et al.*, (1980) *Science* 208:1265; Horsch *et al.*, (1985) *Science* 227: 1229-1231; DeBlock *et al.*, (1989) *Plant Physiology* 91:694-701; *Methods for Plant Molecular Biology* (Weissbach and Weissbach, eds.) Academic Press, Inc. (1988); and *Methods in Plant Molecular Biology* (Schuler and Zielinski, eds.) Academic Press, Inc. (1989). See also, EPA 0193259 and EPA 0451878A1. It is understood that the method of transformation will depend upon the plant cell to be transformed.

It is further recognized that the components of an expression cassette containing the sequence of interest may be modified to increase expression in the plant or plant cell. For example, truncated sequences, nucleotide substitutions or other modifications may be employed. See, for example Perlak *et al.* (1991) *Proc. Natl. Acad. Sci. USA* 88:3324-3328; Murray *et al.* (1989) *Nucleic Acids Research* 17:477-498; and WO 91/16432.

The construct may also include any other necessary regulators such as terminators, (Guerineau *et al.*, (1991), *Mol. Gen. Genet.*, 226:141-144; Proudfoot, (1991), *Cell*, 64:671-674; Sanfacon *et al.*, (1991), *Genes Dev.*, 5:141-149; Mogen *et al.*, (1990), *Plant Cell*, 2:1261-1272; Munroe *et al.*, (1990), *Gene*, 91:151-158; Ballas *et al.*, (1989), *Nucleic Acids Res.*, 17:7891-7903; Joshi *et al.*, (1987), *Nucleic Acid Res.*, 15:9627-9639); plant translational consensus sequences (Joshi, C.P., (1987), *Nucleic Acids Research*, 15:6643-6653), introns (Luehrsen and Walbot, (1991), *Mol. Gen. Genet.*, 225:81-93) and the like,

operably linked to the nucleotide sequence. It may be beneficial to include 5' leader sequences in the expression cassette construct. Such leader sequences can act to enhance translation. Translational leaders are known in the art and include:

Picomavirus leaders, for example, EMCV leader (Encephalomyocarditis 5' noncoding region) (Elroy-Stein, O., Fuerst, T.R., and Moss, B. (1989) PNAS USA 86:6126-6130);

Potyvirus leaders, for example, TEV leader (Tobacco Etch Virus) (Allison *et al.*, (1986); MDMV leader (Maize Dwarf Mosaic Virus); Virology, 154:9-20), and

Human immunoglobulin heavy-chain binding protein (BiP), (Macejak, D.G., and Sarnow, P., (1991), Nature, 353:90-94;

Untranslated leader from the coat protein mRNA of alfalfa mosaic virus (AMV RNA 4), (Jobling, S.A., and Gehrke, L., (1987), Nature, 325:622-625;

Tobacco mosaic virus leader (TMV), (Gallie, D.R. *et al.*, (1989), Molecular Biology of RNA, pages 237-256; and

Maize Chlorotic Mottle Virus leader (MCMV) (Lommel, S.A. *et al.*, (1991), Virology, 81:382-385. See also, Della-Cioppa *et al.*, (1987), Plant Physiology, 84:965-968.

A plant terminator may be utilized in the expression cassette. See, Rosenberg *et al.*, (1987), Gene, 56:125; Guerineau *et al.*, (1991), Mol. Gen. Genet., 226:141-144; Proudfoot, (1991), Cell, 64:671-674; Sanfacon *et al.*, (1991), Genes Dev., 5:141-149; Mogen *et al.*, (1990), Plant Cell, 2:1261-1272; Munroe *et al.*, (1990), Gene, 91:151-158; Ballas *et al.*, (1989), Nucleic Acids Res., 17:7891-7903; Joshi *et al.*, (1987), Nucleic Acid Res., 15:9627-9639.

For tissue specific expression, the nucleotide sequences of the invention can be operably linked to tissue specific promoters. See, for example, US WO 93/07278 herein incorporated by reference.

Further comprised within the scope of the present invention are transgenic plants, in particular transgenic fertile plants transformed by means of the aforescribed processes and their asexual and/or sexual progeny, which still comprises a DNA molecule encoding a monoclonal antibody or a hybrid toxin according to the invention. Mature plants that have been raised from the transformed plant material according to the invention, are either selfed or outcrossed for the production of seeds.

The transgenic plant according to the invention may be a dicotyledonous or a monocotyledonous plant. Preferred are monocotyledonous plants of the *Graminaceae*

family involving Lolium, Zea, Triticum, Triticale, Sorghum, Saccharum, Bromus, Oryzae, Avena, Hordeum, Secale and Setaria plants.

Especially preferred are transgenic maize, wheat, barley, sorghum, rye, oats, turf grasses and rice.

Among the dicotyledonous plants soybean, cotton, tobacco, sugar beet, oilseed rape, and sunflower are especially preferred herein.

The expression 'progeny' is understood to embrace both, "asexually" and "sexually" generated progeny of transgenic plants. This definition is also meant to include all mutants and variants obtainable by means of known processes, such as for example cell fusion or mutant selection and which still exhibit the characteristic properties of the initial transformed plant, together with all crossing and fusion products of the transformed plant material.

Another object of the invention concerns the proliferation material of transgenic plants.

The proliferation material of transgenic plants is defined relative to the invention as any plant material that may be propagated sexually or asexually *in vivo* or *in vitro*. Particularly preferred within the scope of the present invention are protoplasts, cells, calli, tissues, organs, seeds, embryos, pollen, egg cells, zygotes, tubers, grains, fruit, together with any other propagating material obtained from transgenic plants.

Parts of plants, such as for example flowers, stems, fruits, leaves, roots originating in transgenic plants or their progeny previously transformed by means of the process of the invention and therefore consisting at least in part of transgenic cells, are also an object of the present invention.

Before the plant propagation material [fruit, tuber, grains, seed], but especially seed is sold as a commercial product, it is customarily treated with a protectant coating comprising herbicides, insecticides, fungicides, bactericides, nematocides, molluscicides or mixtures of several of these preparations, if desired together with further carriers, surfactants or application-promoting adjuvants customarily employed in the art of formulation to provide protection against damage caused by bacterial, fungal or animal pests.

In order to treat the seed, the protectant coating may be applied to the seeds either by impregnating the tubers or grains with a liquid formulation or by coating them with a

combined wet or dry formulation. In addition, in special cases, other methods of application to plants are possible, eg treatment directed at the buds or the fruit.

The plant seed according to the invention comprising a DNA sequence encoding a monoclonal antibody or a hybrid toxin according to the invention may be treated with a seed protectant coating comprising a seed treatment compound, such as, for example, captan, carboxin, thiram (TMTD®), methalaxyl (Apron®) and pirimiphos-methyl (Actellic®) and others that are commonly used in seed treatment.

It is thus a further object of the present invention to provide plant propagation material for cultivated plants, but especially plant seed that is treated with an seed protectant coating customarily used in seed treatment.

The hybrid toxin proteins of the invention may be used for protecting agricultural crops and products from pests. Alternatively, a gene encoding the hybrid toxin may be introduced via a suitable vector into a microbial host, and said host applied to the environment or plants or animals. Microorganism hosts may be selected which are known to occupy the "phytosphere" (phylloplane, phyllosphere, rhizosphere, and/or rhizoplane) of one or more crops of interest. These microorganisms are selected so as to be capable of successfully competing in the particular environment with the wild-type microorganisms, provide for stable maintenance and expression of the gene expressing the polypeptide pesticide, and, desirably, provide for improved protection of the pesticide from environmental degradation and inactivation.

Such microorganisms include bacteria, algae, and fungi. Of particular interest are microorganisms, such as bacteria, e.g., *Bacillus*, *Caulobacter*, *Agmenellum*, *Pseudomonas*, *Erwinia*, *Serratia*, *Klebsiella*, *Xanthomonas*, *Streptomyces*, *Rhizobium*, *Rhodopseudomonas*, *Methylius*, *Agrobacterium*, *Acetobacter*, *Lactobacillus*, *Arthrobacter*, *Azotobacter*, *Leuconostoc*, and *Alcaligenes*; fungi, particularly yeast, e.g., *Saccharomyces*, *Cryptococcus*, *Kluyveromyces*, *Sporobolomyces*, *Rhodotorula*, and *Aureobasidium*. Of particular interest are such phytosphere bacterial species as *Pseudomonas syringae*, *Pseudomonas fluorescens*, *Serratia marcescens*, *Acetobacter xylinum*, *Agrobacteria*, *Rhodopseudomonas spheroides*, *Xanthomonas campestris*, *Rhizobium melioli*, *Alcaligenes entrophus*, *Clavibacter xyli* and *Azotobacter vinlandii*, and phytosphere yeast species such as *Rhodotorula rubra*, *R. glutinis*, *R. marina*, *R. aurantiaca*, *Cryptococcus albidus*, *C. diffluens*, *C. laurentii*, *Saccharomyces rosei*, *S. pretoriensis*, *S. cerevisiae*, *Sporobolomyces rosues*, *S. odor*,

Kluyveromyces veronae, and *Aureobasidium pollulans*. Of particular interest are the pigmented microorganisms.

The utility of the novel toxin hybrid genes present in a recombinant strain of microorganism is illustrated in Example 7. It should also be recognized that the isolated novel toxin hybrid gene of the present invention can be transferred into any microbial host and confer their insecticidal properties upon that host. Alternate hosts for the novel toxin hybrid gene of the present invention can be selected as suitable for cloning purposes, for purposes of characterizing the form and function of the gene or encoded protein, for use as a fermentation host to increase production of the toxin hybrid protein, for purposes of delivering at least one of the toxin hybrid proteins more effectively to the target insect pest, or introduction of the novel toxin hybrid gene into insect pathogens such as baculovirus [a nuclear polyhedrosis virus, eg [*Autographica californica*] to improve their effectiveness.

The novel toxin hybrid genes or recombinant forms thereof can be transformed into such alternate hosts using a variety of art recognized methods. One such preferred method is electroporation of microbial cells, as described, for example, by the method of Dower (U.S. Patent No. 5,186,800). Another preferred method is that of Schurter et al. (Mol. Gen. Genet. 218: 177-181 (1989)), which is also disclosed in EP-A 0 342 633 which is incorporated herein in its entirety.

It is envisioned that said alternate host would be applied to the environment or plants or animals for insect control. Microorganism hosts may be selected which are known to occupy the "phytosphere" (phylloplane, phyllosphere, rhizosphere, and/or rhizoplane) of one or more crops of interest. These microorganisms are selected so as to be capable of successfully competing in the particular environment with the wild-type microorganisms, provide for stable maintenance and expression of the gene expressing the polypeptide pesticide, and, desirably, provide for improved protection of the pesticide from environmental degradation and inactivation.

The present invention further provides an entomocidal composition comprising as an active ingredient at least one of the novel toxin hybrids according to the invention or a recombinant microorganism containing at least one of the novel toxin genes in recombinant form together with an agricultural adjuvant such as a carrier, diluent, surfactant or application-promoting adjuvant. The composition may also contain a further biologically active compound. The said compound can be both a fertilizer or micronutrient

donor or other preparations that influence plant growth. It can also be a selective herbicide, insecticide, fungicide, bactericide, nematocide, molluscicide or mixtures of several of these preparations, if desired, together with further agriculturally acceptable carriers, surfactants or application-promoting adjuvants customarily employed in the art of formulation. Suitable carriers and adjuvants can be solid or liquid and correspond to the substances ordinarily employed in formulation technology, e.g. natural or regenerated mineral substances, solvents, dispersants, wetting agents, tackifiers, binders or fertilizers

The composition may comprise from 0.1 to 99% by weight of the active ingredient, from 1 to 99.9% by weight of a solid or liquid adjuvant, and from 0 to 25% by weight of a surfactant. The active ingredient comprising at least one of the novel toxin hybrid toxins according to the invention or a recombinant microorganism containing at least one of the novel toxin genes in recombinant form or the composition containing the said active ingredient, may be administered to the plants or crops to be protected together with certain other insecticides or chemicals (1993 Crop Protection Chemicals Reference, Chemical and Pharmaceutical Press, Canada) without loss of potency. It may be administered as a dust, a suspension, a wettable powder or in any other material form suitable for agricultural application.

The invention further provides methods for controlling or inhibiting of insect pests by applying an active ingredient comprising at least one of the novel toxin hybrids according to the invention or a recombinant microorganism containing at least one of the novel toxin hybrid gene in recombinant form or a composition comprising the said active ingredient to (a) an environment in which the insect pest may occur, (b) a plant or plant part in order to protect said plant or plant part from damage caused by an insect pest, or (c) seed in order to protect a plant which develops from said seed from damage caused by an insect pest.

A preferred method of application in the area of plant protection is application to the foliage of the plants (foliar application), with the number of applications and the rate of application depending on the plant to be protected and the risk of infestation by the pest in question. However, the active ingredient may also penetrate the plants through the roots (systemic action) if the locus of the plants is impregnated with a liquid formulation or if the active ingredient is incorporated in solid form into the locus of the

plants, for example into the soil, e.g. in granular form (soil application). In paddy rice crops, such granules may be applied in metered amounts to the flooded rice field.

The compositions according to the invention are also suitable for protecting plant propagating material, e.g. seed, such as fruit, tubers or grains, or plant cuttings, from insect pests. The propagation material can be treated with the formulation before planting: seed, for example, can be dressed before being sown. The active ingredient of the invention can also be applied to grains (coating), either by impregnating the grains with a liquid formulation or by coating them with a solid formulation. The formulation can also be applied to the planting site when the propagating material is being planted, for example to the seed furrow during sowing. The invention relates also to those methods of treating plant propagation material and to the plant propagation material thus treated.

The compositions according to the invention comprising as an active ingredient a recombinant microorganism containing at least one of the novel toxin hybrid genes in recombinant form may be applied in any method known for treatment of seed or soil with bacterial strains. For example, see US Patent No. 4,863,866. The strains are effective for biocontrol even if the microorganism is not living. Preferred is, however, the application of the living microorganism.

Target crops to be protected within the scope of the present invention comprise, e.g., the following species of plants:

cereals (wheat, barley, rye, oats, rice, sorghum and related crops), beet (sugar beet and fodder beet), forage grasses (orchardgrass, fescue, and the like), drupes, pomes and soft fruit (apples, pears, plums, peaches, almonds, cherries, strawberries, raspberries and blackberries), leguminous plants (beans, lentils, peas, soybeans), oil plants (rape, mustard, poppy, olives, sunflowers, coconuts, castor oil plants, cocoa beans, groundnuts), cucumber plants (cucumber, marrows, melons) fiber plants (cotton, flax, hemp, jute), citrus fruit (oranges, lemons, grapefruit, mandarins), vegetables (spinach, lettuce, asparagus, cabbages and other Brassicae, onions, tomatoes, potatoes, paprika), lauraceae (avocados, carrots, cinnamon, camphor), deciduous trees and conifers (e.g. linden-trees, yew-trees, oak-trees, alders, poplars, birch-trees, firs, larches, pines), or plants such as maize, tobacco, nuts, coffee, sugar cane, tea, vines, hops, bananas and natural rubber plants, as well as ornamentals (including composites).

A recombinant microorganism containing at least one of the novel gene in recombinant form is normally applied in the form of entomocidal compositions and can be applied to the crop area or plant to be treated, simultaneously or in succession, with further biologically active compounds. These compounds may be both fertilizers or micronutrient donors or other preparations that influence plant growth. They may also be selective herbicides, insecticides, fungicides, bactericides, nematocides, molluscicides or mixtures of several of these preparations, if desired together with further carriers, surfactants or application-promoting adjuvants customarily employed in the art of formulation.

The active ingredient according to the invention may be used in unmodified form or together with any suitable agriculturally acceptable carrier. Such carriers are adjuvants conventionally employed in the art of agricultural formulation, and are therefore formulated in known manner to emulsifiable concentrates, coatable pastes, directly sprayable or dilutable solutions, dilute emulsions, wettable powders, soluble powders, dusts, granulates, and also encapsulations, for example, in polymer substances. Like the nature of the compositions, the methods of application, such as spraying, atomizing, dusting, scattering or pouring, are chosen in accordance with the intended objective and the prevailing circumstances. Advantageous rates of application are normally from about 50 g to about 5 kg of active ingredient (a.i.) per hectare ("ha", approximately 2.471 acres), preferably from about 100 g to about 2kg a.i./ha. Important rates of application are about 200 g to about 1kg a.i./ha and 200g to 500g a.i./ha.

For seed dressing advantageous application rates are 0.5 g to 1000 g a.i. per 100 kg seed, preferably 3 g to 100 g a.i. per 100 kg seed or 10 g to 50 g a.i. per 100 kg seed.

Suitable carriers and adjuvants can be solid or liquid and correspond to the substances ordinarily employed in formulation technology, e.g. natural or regenerated mineral substances, solvents, dispersants, wetting agents, tackifiers, binders or fertilizers. The formulations, i.e. the entomocidal compositions, preparations or mixtures containing the recombinant microorganism containing the novel gene in recombinant form as an active ingredient or combinations thereof with other active ingredients, and, where appropriate, a solid or liquid adjuvant, are prepared in known manner, e.g., by homogeneously mixing and/or grinding the active ingredients with extenders, e.g., solvents, solid carriers, and in some cases surface-active compounds (surfactants).

Suitable solvents are: aromatic hydrocarbons, preferably the fractions containing 8 to 12 carbon atoms, e.g. xylene mixtures or substituted naphthalenes, phthalates such as dibutyl phthalate or dioctyl phthalate, aliphatic hydrocarbons such as cyclohexane or paraffins, alcohols and glycols and their ethers and esters, such as ethanol, ethylene glycol monomethyl or monoethyl ether, ketones such as cyclohexanone, strongly polar solvents such as N-methyl-2-pyrrolidone, dimethylsulfoxide or dimethylformamide, as well as vegetable oils or epoxidised vegetable oils such as epoxidised coconut oil or soybean oil; or water.

The solid carriers used, e.g., for dusts and dispersible powders, are normally natural mineral fillers such as calcite, talcum, kaolin, montmorillonite or attapulgite. In order to improve the physical properties it is also possible to add highly dispersed silicic acid or highly dispersed absorbent polymers. Suitable granulated adsorptive carriers are porous types, for example pumice, broken brick, sepiolite or bentonite; and suitable nonsorbent carriers are materials such as calcite or sand. In addition, a great number of pregranulated materials of inorganic or organic nature can be used, e.g. especially dolomite or pulverized plant residues.

Depending on the nature of the active ingredients to be formulated, suitable surface-active compounds are non-ionic, cationic and/or anionic surfactants having good emulsifying, dispersing and wetting properties. The term "surfactants" will also be understood as comprising mixtures of surfactants. Suitable anionic surfactants can be both water-soluble soaps and water-soluble synthetic surface-active compounds. Suitable soaps are the alkali metal salts, alkaline earth metal salts or unsubstituted or substituted ammonium salts of higher fatty acids (C_{10} - C_{22}), e.g. the sodium or potassium salts of oleic or stearic acid, or of natural fatty acid mixtures which can be obtained, e.g. from coconut oil or tallow oil. Further suitable surfactants are also the fatty acid methyltaurin salts as well as modified and unmodified phospholipids.

More frequently, however, so-called synthetic surfactants are used, especially fatty sulfonates, fatty sulfates, sulfonated benzimidazole derivatives or alkylarylsulfonates. The fatty sulfonates or sulfates are usually in the forms of alkali metal salts, alkaline earth metal salts or unsubstituted or substituted ammonium salts and generally contain a C_{8} - C_{22} alkyl radical which also includes the alkyl moiety of acyl radicals, e.g. the sodium or calcium salt of lignosulfonic acid, of dodecylsulfate, or of a mixture of fatty alcohol sulfates obtained from natural fatty acids. These compounds also comprise the

salts of sulfuric acid esters and sulfonic acids of fatty alcohol/ethylene oxide adducts. The sulfonated benzimidazole derivatives preferably contain 2 sulfonic acid groups and one fatty acid radical containing about 8 to 22 carbon atoms. Examples of alkylarylsulfonates are the sodium, calcium or triethanolamine salts of dodecylbenzenesulfonic acid, dibutyl-naphthalenesulfonic acid, or of a naphthalenesulfonic acid/formaldehyde condensation product. Also suitable are corresponding phosphates, e.g. salts of the phosphoric acid ester of an adduct of p-nonylphenol with 4 to 14 moles of ethylene oxide.

Non-ionic surfactant are preferably polyglycol ether derivatives of aliphatic or cycloaliphatic alcohols, or saturated or unsaturated fatty acids and alkylphenols, said derivatives containing 3 to 30 glycol ether groups and 8 to 20 carbon atoms in the (aliphatic) hydrocarbon moiety and 6 to 18 carbon atoms in the alkyl moiety of the alkylphenols.

Further suitable non-ionic surfactants are the water-soluble adducts of polyethylene oxide with polypropylene glycol, ethylenediaminopolypropylene glycol and alkylpolypropylene glycol containing 1 to 10 carbon atoms in the alkyl chain, which adducts contain 20 to 250 ethylene glycol ether groups and 10 to 100 propylene glycol ether groups. These compounds usually contain 1 to 5 ethylene glycol units per propylene glycol unit. Representative examples of non-ionic surfactants are nonylphenolpolyethoxyethanols, castor oil polyglycol ethers, polypropylene/polyethylene oxide adducts, tributylphenoxypolyethoxyethanol, polyethylene glycol and octylphenoxypolyethoxyethanol. Fatty acid esters of polyoxyethylene sorbitan, such as polyoxyethylene sorbitan trioleate, are also suitable non-ionic surfactants.

Cationic surfactants are preferably quaternary ammonium salts which contain, as N-substituent, at least one C₈-C₂₂ alkyl radical and, as further substituents, lower unsubstituted or halogenated alkyl, benzyl or hydroxyl-lower alkyl radicals. The salts are preferably in the form of halides, methylsulfates or ethylsulfates, e.g., stearyltrimethylammonium chloride or benzyldi-(2-chloroethyl)ethylammonium bromide.

The surfactants customarily employed in the art of formulation are described, e.g., in "McCutcheon's Detergents and Emulsifiers Annual", MC Publishing Corp. Ridgewood, N.J., 1979; Dr. Helmut Stache, "Tensid Taschenbuch" (Handbook of Surfactants), Carl Hanser Verlag, Munich/Vienna.

Another particularly preferred characteristic of an entomocidal composition of the present invention is the persistence of the active ingredient when applied to plants and soil. Possible causes for loss of activity include inactivation by ultra-violet light, heat, leaf exudates and pH. Formulation of an entomocidal composition of the present invention can address these problems by either including additives to help prevent loss of the active ingredient or encapsulating the material in such a way that the active ingredient is protected from inactivation. Encapsulation can be accomplished chemically (McGuire and Shasha, J Econ Entomol 85: 1425-1433, 1992) or biologically (Barnes and Cummings, 1986; EP-A 0 192 319). Chemical encapsulation involves a process in which the active ingredient is coated with a polymer while biological encapsulation involves the expression of the hybrid toxin genes in a microbe. For biological encapsulation, the intact microbe containing the hybrid toxin protein is used as the active ingredient in the formulation. The addition of UV protectants might effectively reduce irradiation damage. Inactivation due to heat could also be controlled by including an appropriate additive.

Preferred within the present application are formulations comprising living microorganisms as active ingredient either in form of the vegetative cell or more preferable in form of spores, if available. Suitable formulations may consist, for example, of polymer gels which are crosslinked with polyvalent cations and comprise these microorganisms. This is described, for example, by D.R. Fravel et al. in *Phytopathology*, Vol. 75, No. 7, 774-777, 1985 for alginate as the polymer material. It is also known from this publication that carrier materials can be co-used. These formulations are as a rule prepared by mixing solutions of naturally occurring or synthetic gel-forming polymers, for example alginates, and aqueous salt solutions of polyvalent metal ions such that individual droplets form, it being possible for the microorganisms to be suspended in one of the two or in both reaction solutions. Gel formation starts with the mixing in drop form. Subsequent drying of these gel particles is possible. This process is called ionotropic gelling. Depending on the degree of drying, compact and hard particles of polymers which are structurally crosslinked via polyvalent cations and comprise the microorganisms and a carrier present predominantly uniformly distributed are formed. The size of the particles can be up to 5 mm.

Compositions based on partly crosslinked polysaccharides which, in addition to a microorganism, for example, can also comprise finely divided silicic acid as the carrier material, crosslinking taking place, for example, via Ca^{++} ions, are described in EP-A1-0

097 571. The compositions have a water activity of not more than 0.3. W.J. Cornick et al. describe in a review article [New Directions in Biological Control: Alternatives for Suppressing Agricultural Pests and Diseases, pages 345-372, Alan R. Liss, Inc. (1990)] various formulation systems, granules with vermiculite as the carrier and compact alginate beads prepared by the ionotropic gelling process being mentioned. Such compositions are also disclosed by D.R. Fravel in Pesticide Formulations and Application Systems: 11th Volume, ASTM STP 1112 American Society for Testing and Materials, Philadelphia, 1992, pages 173 to 179 and can be used to formulate the recombinant microorganisms according to the invention.

The entomocidal compositions of the invention usually contain from about 0.1 to about 99%, preferably about 0.1 to about 95%, and most preferably from about 3 to about 90% of the active ingredient, from about 1 to about 99.9%, preferably from about 1 to about 99%, and most preferably from about 5 to about 95% of a solid or liquid adjuvant, and from about 0 to about 25%, preferably about 0.1 to about 25%, and most preferably from about 0.1 to about 20% of a surfactant.

In a preferred embodiment of the invention the entomocidal compositions usually contain 0.1 to 99%, preferably 0.1 to 95%, of a recombinant microorganism containing at least one of the novel genes in recombinant form, or combination thereof with other active ingredients, 1 to 99.9% of a solid or liquid adjuvant, and 0 to 25%, preferably 0.1 to 20%, of a surfactant.

Whereas commercial products are preferably formulated as concentrates, the end user will normally employ dilute formulations of substantially lower concentration. The entomocidal compositions may also contain further ingredients, such as stabilizers, antifoams, viscosity regulators, binders, tackifiers as well as fertilizers or other active ingredients in order to obtain special effects.

A number of ways are available for introducing a gene expressing the hybrid toxin into the microorganism host under conditions which allow for stable maintenance and expression of the gene. For example, expression cassettes can be constructed which include the DNA constructs of interest operably linked with the transcriptional and translational regulatory signals for expression of the DNA constructs, and a DNA sequence homologous with a sequence in the host organism, whereby integration will occur, and/or a replication system which is functional in the host, whereby integration or stable maintenance will occur.

Transcriptional and translational regulatory signals include but are not limited to promoter, transcriptional initiation start site, operators, activators, enhancers, other regulatory elements, ribosomal binding sites, an initiation codon, termination signals, and the like. See, for example, US Patent 5,039,523; US Patent No. 4,853,331; EPO 0480762A2; Sambrook *et al. supra*; Molecular Cloning, a Laboratory Manual, Maniatis *et al. (eds)* Cold Spring Harbor Laboratory, Cold Spring Harbor, NY (1982); Advanced Bacterial Genetics, Davis *et al. (eds.)* Cold Spring Harbor Laboratory, Cold Spring Harbor, NY (1980); and the references cited therein.

The following examples are offered by way of illustration and are not meant to be limiting on the invention described herein.

EXPERIMENTAL

EXAMPLE 1: MONOCLONAL ANTIBODY DEVELOPMENT

1.1. IMMUNIZATION:

Appropriate quantities of the antigen (approximately 50 micrograms of corn rootworm BBMV) were emulsified in a non-oil based adjuvant and used to immunize a group of ten Balb/c mice. Mice were given booster immunizations at biweekly intervals. Seven days following the third booster injection, serum samples were taken from the mice, and the relative serum antibody titers determined by an enzyme-linked immunosorbent assay (ELISA), as described below. A group of four mice with the highest titers were given a final regimen of low-dose antigen boosters (approximately one-tenth of the dosage used during the regular immunization) over a period of three days, and used as spleen donors in fusions as described below.

1.2. FUSION:

Four mice with specific antibody titers of $>1:5,000$ were selected for two fusions. Spleens were aseptically excised, mechanically dissociated and the lymphocytes isolated as follows. The red blood cells were lysed by incubation with a 0.155 M solution of ammonium chloride in 0.017 M TRIZMA base, pH 7.2 (Sigma Chemical Company, St. Louis, MO). The cells were washed twice with phosphate buffered saline (PBS) and

lymphocytes further purified by running a density gradient centrifugation as follows. Cells were carefully layered on a Ficoll (Sigma Chemical Company, St. Louis, MO) solution of specific gravity 1.065 (Van Mourik *et al.*, *Meth. in Enzymology*, 121:174-182 (1986)) and centrifuged at 450 x g for 20 minutes. The pellet that contains cells of density greater than 1.065 is greatly enriched in lymphocytes, and was fused with myeloma cells.

A polyethylene glycol-mediated fusion was performed using isolated lymphocytes and a Balb/c-derived HGPRT (Hypoxanthine Guanine Phosphoribosyl Transferase) deficient SP2/0 plasmacytoma cell line. Lymphocytes were mixed with myeloma cells at a ratio of 4:1. The cell mixture was thoroughly mixed, centrifuged and the fusion carried out as follows (Oi *et al.* in *Selected Methods in Cellular Immunology* eds. Michell, B.B. and Shiigi, S.M. (Freeman, San Francisco) 351-371 (1980), Fazekas *et al.*, *J. Immunol. Meth.*, 35:1-21 (1980)). The cell pellet was carefully suspended in 1 ml of a 50% polyethylene glycol (PEG) with constant stirring over a period of one minute. The PEG concentration was gradually reduced by diluting the cells with serum free RPMI media (Gibco BRL, Gaithersburg, MD). After the fusion, the fused cells were pelleted at 80 x g for 5 minutes, resuspended and plated into 96 well plates at a density of 10^5 to 10^6 total cells per well. Spleen feeder cells, prepared by treating non-immune splenocytes with 20 $\mu\text{g/ml}$ mitomycin C were added to provide supplemental growth factors to the fused cells. For the next several days, the hybridomas were selected by using HAT (Hypoxanthine Aminopterin Thymidine) media that contained 17.6 $\mu\text{g/ml}$ of aminopterin. Growing colonies were seen under an inverted microscope as early as 3 or 4 days after fusion. However, macroscopic colonies were not visible until 10 to 14 days after fusion. At this stage, the supernatants of each well were assayed for specific antibody secretion.

1.3. SCREENING:

Upon detection of viable hybridoma colonies surviving HAT selection, the supernatants were screened using an enzyme-linked immunosorbent assay (ELISA) (Engvall, *Meth. Enzymology*, 70:419 (1980), Engvall *et al.*, *Immunochemistry*, 8:871 (1971)). Very briefly, hybridoma supernatants were incubated in wells of 96-well microtiter plates bearing approximately 500 nanograms per well of the antigen. After appropriate washing steps, as described in Engvall *et al.* reference, bound antibodies were identified using a goat anti-mouse second antibody conjugated to horseradish peroxidase (HRP). After additional washing steps, the enzyme activity in each well was

quantified with chromogenic substrate. The resultant absorbance at 492 nm (OD₄₉₂) was measured with an automated ELISA reader to identify the positive colonies. Hybridoma lines with strong binding to the corn rootworm BBMV were further screened by three additional ELISA screens to eliminate those monoclonals which bind to either mammalian or plant proteins. More specifically, ELISAs were performed using rabbit intestinal brush border membranes and maize leaf and root microsomal membrane preparations. An ELISA screen to identify lines with cross-reactivity to European corn borer BBMV was also included.

Hybridoma colonies which secreted antibodies which bound to the corn rootworm BBMV antigen and not to the mammalian and plant proteins were cloned as described below.

1.4. CLONING:

In this phase, hybridomas which secrete antibodies with apparent specificity for the antigen were expanded and cloned in 96 well plates at target concentrations of 0.5, 1 and 5 cells/well. Growth factors (Sugasawara *et al.*, J. Immunol. Meth., 79:276-275 (1985)) were provided to promote hybridoma cells grown from limited densities. After 2-3 weeks, when the clones were large enough, positive clones were identified again using the ELISAs as described under "Screening." Representative clones were then expanded for antibody production.

1.5. ASCITES PRODUCTION:

Large-scale production of appropriate monoclonal antibodies was accomplished by growing the hybridomas as ascites tumors in pristane-primed Balb/c mice (Brodeur *et al.*, J. Immunol. Meth., 71:265-272 (1984)). Ascites fluid was pooled, and the antibodies partially purified from dialyzed ascites by Protein A chromatography. The resultant antibody preparations were aliquoted and frozen.

Cell lines which produce monoclonal antibodies useful in the present invention are described in Table 1. The hybridoma cell lines whose ATCC designations are indicated in Table 1 were deposited with the American Type Culture Collection 12301 Parklawn Drive, Rockville, MD 20852 USA on April 19, 1994.

TABLE 1
CRW BBMV MONOCLONAL LINES

Cell line	ECB Crossreact	Western Blot	Isotyp e	ATCC Designati on
1A4	yes	2 CRW bands	IgM-k	
1A11	no	2 CRW bands	IgM-k	
1F51	yes	2 CRW bands	IgM-k	
2B5	yes	2 CRW bands	IgM-k	HB 11619
3B1	no	1 CRW band	IgG1-k	HB 11617
7G6	no	> 5 bands	IgM-k	
10A1	no	no signal	IgM-k	
10B6	no	> 5 bands C	IgG3- K	HB 11618
10F9	no	> 5 bands B	IgM-k	
12G4	no	not done	IgM-k	
14G1	no	>5 bands	IgG2B -k	
17F6	no	>5 bands A	IgG1-k	HB 11620
17H6	no	>5 bands A	IgG2A -k	
18A7	no	>5 bands B	IgG1-k	
16E4	yes	>5 bands	IgM	HB 11616

EXAMPLE 2: ISOLATION OF BRUSH BORDER MEMBRANES VESICLES (BBMV)**2.1. ISOLATION OF CORN ROOTWORM BBMV:**

Vesicles were prepared based on the method of Wolfersberger *et al.*, Comp. Biochem. Physiol. 86A:301-308 (1987) as modified by English and Readdy (Insect Biochem. 19;145-152 (1989)). The guts of third instar corn rootworm larvae were homogenized in ice-cold 50 mM sucrose, 2 mM Tris-Cl (pH 7.4), 0.1 mM phenylmethylsulfonyl fluoride using a Potter-Elvehjem homogenizer. Calcium chloride was added to 10 mM and the homogenate stirred on ice for 15 min. The homogenate was centrifuged at 4,300 x g for 10 min at 4°C, and the pellet discarded. The supernatant was centrifuged at 27,000 x g for 10 min, and the pellet resuspended in 0.32 M sucrose. The suspension was passed through a 27-gauge needle and stored at -70°C.

2.2. ISOLATION OF EUROPEAN CORNBORER BBMV:

Guts were excised from fifth instar European corn borer (ECB) and cut longitudinally to remove the food contents and peritrophic membrane. Isolation of (ECB) BBMVs were performed using the methods described above.

2.3. ISOLATION OF PLANT MICROSOMES:

Leaf or root tissue from corn plants (grown 72 hrs in dark at 28°C) were ground up with a mortar and pestle in an equal volume of 0.3 M potassium phosphate pH 7.4, 5 mM DTT, and 1% (w/v) PVPP. The mixture was strained through four layers of cheesecloth, followed by centrifugation at 10,000 x g for 15 min. The supernatant was centrifuged at 100,000 x g for 60 min and the pellet resuspended in 0.1 M potassium phosphate pH 7.4.

2.4. ISOLATION OF MAMMALIAN INTESTINAL BBMV:

Mammalian brush border membranes were prepared from the mucosal surface of rabbit duodenum and stroma (Kessler *et al.* BBA 506:136-154 (1978)) using a similar process to the isolation of brush border membrane vesicles from insect guts. The mucosal lining of fresh rabbit duodenum and lower stomach was washed and the mucosal layer separated from the underlying stroma. The material was suspended in a

10-fold volume of ice-cold 50 mM sucrose, 0.1 mM PMSF, 2 mM Tris-HCl (pH7.4) and homogenized with 15 strokes on ice. Calcium chloride was added to a final concentration of 10 mM, and the mixture stirred on ice for 15 min. The homogenate was centrifuged at 4300 x g for 10 min at 4°C. The supernatant was harvested and centrifuged at 27,000 x g for 10 min. The pellet was resuspended in 0.32 M sucrose and frozen at -70°C.

EXAMPLE 3: CHARACTERIZATION OF CRW BBMV MONOCLONAL LINES

Monoclonal lines with strong binding to CRW BBMVs based on ELISA were further screened to select clones with no cross-reactivity to either maize or mammalian microsomes. The additional ELISAs were performed using corn leaf and root microsomes and rabbit intestinal membrane vesicles. All lines were simultaneously screened against European corn borer BBMV proteins. From seventy-eight lines screened, eleven specific to corn rootworm were isolated. In addition to the eleven CRW BBMV-specific lines, four lines with cross-reactivity to ECB BBMVs were isolated. The fifteen lines represent monoclonal lines secreting IgG1, Ig2a, IgG2b, IgG3 and IgM. Monoclonal lines were analyzed by western blot to confirm CRW specificity and absence of cross-reactivity to either rabbit or corn microsomes. The specific binding of the antibodies to various CRW BBMV proteins was also characterized and shown in Figure 1. Five distinct binding patterns were found; two patterns were specific for one or two proteins and the other three represent binding to 7 -15 proteins. The class with 7-15 proteins was further subclassed into A, B, C based on binding pattern.

3.1. WESTERN ANALYSIS OF MONOCLONAL LINES:

Brush border membrane vesicles were prepared as described above and electrophoresed on 8-16 % acrylamide SDS protein gels (Novex, San Diego, CA). Proteins were transferred onto nitrocellulose, (Burnette, W.N. , Western Blotting, 112:195 (1981)) and allowed to bind the supernatant of hybridoma lines. Binding of antibodies to blotted proteins was visualized using standard methods (see, for example, Antibodies: A Laboratory Manual, E. Harlow and D. Lane, Cold Spring Harbor, 1988, and references cited therein).

EXAMPLE 4: CLONING CRW BINDING ANTIBODY DOMAINS

Various methods are known for obtaining corn rootworm specific antibody genes. One method is to clone a random library of antibody genes in a phage and screen the library for ability to bind to corn rootworm gut (CRW) proteins. Another available approach is to generate monoclonal antibodies which bind to CRW gut proteins and then clone the antibody genes from such lines. For the present example, the second method is used. Antibody genes can be cloned from hybridoma cells using primers to conserved DNA sequences within the constant regions and the framework regions of the variable regions and amplified for cloning using the polymerase chain reaction (PCR). See generally, Mullis *et al.*, *Meth. Enzymol.*, 155:335-350 (1987); Erlich, (ed.), *PCR Technology*, Stockton Press (New York 1989). A database of mouse heavy chain and light chain sequences compiled by Kabat *et al.*, US Dept Health and Human Services, US Government Printing Offices (1987) has been successfully used to generate both isotype specific and degenerate primers for cloning antibody genes. (Jones *et al.* *Bio/technology* 9:88-89 (1991)). Additionally, techniques are well known for cloning of smaller fragments of antibodies (Fab) which possess the binding properties of the original antibody. Complete antibodies are large molecules (150 kDa), but much smaller Fab and Fv antigen-binding fragments (12 kDa-50 kDa) have been shown to retain full binding affinity. Single chain Fv fragments (scFv) in which Vh and Vl domains are linked by a hydrophilic and flexible peptide have been used successfully to target enzymes and toxins to specific cells (Bird, *Science* 423:423-426 (1988); Huston, *PNAS* 85:5879-5883 (1988)). Single Vh domains (Dabs) and single complementary determining regions as small as 20 amino acids in length, called minimal recognition units (m.r.u.), have also been used for antigen binding (Ward, *Nature* 341:544-546 (1989); Taub, *J. Biol. Chem* 264:259-265 (1989); Williams, *PNAS* 86:5537-5541 (1989)). Thus, it is possible to reduce the CRW specific binding domain to a very small size.

4.1. CLONING ANTIBODY GENES BY PCR:

Polymerase chain reaction technology and specific oligonucleotide primers were used to clone immunoglobulin genes or regions from immunoglobulin genes. PCR primers specific for both the heavy and light chains of IgM and the three IgG isotypes were selected from the Kabat database described above. Primers for the region encoding the NH₂-terminal end of the mature variable region were designed to initiate at the first framework region and were made with some degeneracy to allow these to be used as

"universal primers". The 3' primers used for the specific PCR amplification of the variable regions were designed from conserved sequences of the first constant domain (CH1) of both the light and heavy chains. A different 3' primer was used for immunoglobulin isotypes IgG1 (3B1 and 17F6), IgG3 (10B6), and IgM (2B5). Isotypes IgG2A and IgG2B can be amplified with the same primers used for IgG1. Antibody variable regions were cloned into a light (pCIB4612) and heavy (pCIB4611) chain expression vector containing an endoplasmic reticulum signal peptide and the constant regions of IgG1 light and heavy chains, respectively.

Table 2 shows the structure of the primers used for the PCR cloning of the mouse immunoglobulin light and heavy variable regions. Alternatively, primer sequences can be used that are available in the published literature (Coloma *et al.* Bio/Techniques 11: 152-156, 1991; Jones *et al.* Bio/Technology 9:88-89, 1991). Oligonucleotides were made on an Applied Biosystems DNA synthesizer 380B (Applied Biosystems, Foster City, CA) using standard conditions as described below. The PCR primers incorporate restriction sites and, after amplification and digestion, were cloned into a plant expression vector under the control of the CaMV 35S promoter. Restriction sites were chosen that were known to be absent in sequenced antibody genes.

TABLE 2

PCR PRIMERS USED FOR AMPLIFICATION OF ANTIBODY GENES

3B1, 2B5, 10B6, 14G1 and 17F6 Light Chain Variable Regions in pCIB4614, pCIB4616, pCIB4625, pCIB4636 and pCIB4617:

NC92: 5' Primer 5'-GTC TCG AGG AYA TYS WGM TSA CCC ART CT-3'
(SEQ ID NO:37)

NC130: 3' Primer 5'-GCA GAT CTA GTT GGT GCA GCA TCA GCC CG-3'
(SEQ ID NO:38)

3B1 and 17F6 Heavy Chain Variable Region in pCIB4613 and pCIB4609:

NC91: 5' Primer 5'-GTC TCG AGC AGG TSM ARC TGC AGS AGT CWG-3'
(SEQ ID NO:39)

NC114: 3' Primer 5'-GCA GAT CTA GAT CCA GGG GCC AGT GGA TA-3'
(SEQ ID NO:40)

2B5 Heavy Chain Variable Region in pCIB4615:

NC91: 5' Primer 5'-GTC TCG AGC AGG TSM ARC TGC AGS AGT CWG-3'
(SEQ ID NO:39)

NC111: 3' Primer 5'-GCA GAT CTG CAG GAG ACG AGG GGG AAG ACA TT-3'
(SEQ ID NO:41)

10B6 Heavy Chain Variable Region in pCIB4637:

DB91: 5' Primer 5'-ACG TCT CGA GGA RGT GAA GCT KRW KGA RWC TG-3'
(SEQ ID NO:48)

NC117: 3' Primer 5'-GCA GAT CTG CAG CCA GGG ACC AAG GGA TA-3'

(SEQ ID NO:42)

14G1 Heavy Chain Variable Region in pCIB4635:

DB91: 5' Primer 5'-ACG TCT CGA GGA RGT GAA GCT KRW KGA RWC TG-3'

(SEQ ID NO:48)

DB114: 3' Primer 5'-CAA TTC GCA TAT GAG ATC CAG GGG CCA GTG GAT A-3'

(SEQ ID NO:49)

Y= C or T; S= C or G; W= A or T; M= C or A; R= A or G

Poly-A⁺ RNA isolated from hybridoma lines was used to generate first strand cDNA for subsequent use in PCR reactions. Poly-A⁺ RNA was extracted from 10⁸ hybridoma cells using a procedure based on guanidinium thiocyanate lysis and oligo (dT) cellulose purification using the Fast Track mRNA Isolation Kit, (Invitrogen Corp., San Diego, CA). Approximately one tenth of the RNA isolated from 10⁸ cells (or ~500 ng) was used to generate first strand cDNA. RNA was incubated at 42 C for 30 min then heated to 95 C for 5 min with a mixture of deoxynucleotides (0.2 mM each dNTP), 5 µg random hexamer pd (N6) (Pharmacia LKB Biotechnology Inc., Piscataway, NJ.) as primer, 50 units Moloney Murine Leukemia Virus reverse transcriptase (Pharmacia LKB Biotechnology Inc., Piscataway, NJ) and 1X PCR buffer in a 100 µl reaction volume. The first-strand cDNA reaction was extracted with phenol-chloroform and centrifuged through a size exclusion spin column (Chroma Spin 30, Clontech Laboratories, Inc., Palo Alto, CA) to remove random hexamers. Next, one tenth (or 10 µl) of the first-strand cDNA reaction was added to a 50 µl PCR reaction mix containing immunoglobulin specific primers following the instructions of the Perkin-Elmer Cetus Amplification Kit. The mixture was amplified using the Perkin-Elmer Cetus Thermal Cycler for 20 cycles. The temperature and times used for PCR were as follows: denaturation at 94 C for 1 min; annealing at 52 C for 1 min 30 sec; extension at 72 C for 1 min. PCR products were electrophoresed on 6% acrylamide gels (Novex, Encinitas, CA) and DNA purified from gel slices. Gel slices were crushed in 200 µl TE and purified by centrifugation through Ultrafree-MC Millipore columns (Millipore, Bedford, MA). Eluate was treated with 50 µg/ml proteinase K at 37 C for 30 min, extracted with phenol-chloroform-isoamyl (50:48:2), followed by an additional chloroform extraction and ethanol precipitated. DNA was resuspended in 40 µl TE and digested with appropriate restriction enzymes. PCR products of antibody variable regions were digested with Xho I and Bgl II and re-purified on 6% acrylamide gels as described above. Final Xho I/Bgl II fragments were ligated to either the light (pCIB4612) or the heavy (pCIB4611) antibody chain expression vector digested with Xho I and Bgl II. Expression vector pCIB4612 contains the CaMV 35S promoter and termination sequence with a 19 amino acid signal peptide sequence and the light chain constant region CH1. Variable light chain regions were cloned into the Xho I/ Bgl II site for expression of a full length light chain.

All antibody genes were cloned by the above procedure except the heavy chain of 10B6 and the heavy and light chains of 14G1. These antibody genes were cloned from

PCR products, but the products were separated by electrophoresis on 6% acrylamide TBE gels, the fragments cut out of the gel and eluted into 0.7 M LiCl plus 2 mM EDTA. The fragments were precipitated and resuspended in 10 mM Tris plus 2 mM EDTA, pH 7.5. The isolated PCR products were ligated directly into a pUC derived cloning vector, pT&Blue T (Novagen, Inc.). Since Taq DNA polymerase leaves a single 3' A-nucleotide overhang on the reaction products (Clark, Nucl. Acids Res. 16: 9677 (1988)), these products can be cloned directly into a vector containing compatible single T-nucleotide overhangs (Marchuk et al. Nucl. Acids Res. 19: 1154 (1990)).

The pCIB4612 vector was made by ligating a 155 base pair Dde I /Sty I light chain constant region from a mouse Ig Kappa chain (Schulze-Gahmen *et al.* 1988, J. Biol. Chem. 263:17100-17106; Kabat *et al.*, US Dept Health and Human Services, US Government Printing Offices (1987)) in a four way ligation to a 71 bp Xho I /Dde I fragment, a 101 bp Sty I /Bgl II fragment, and a 3.8 Kb Xho I/Bgl II vector fragment from pCIB4610. Oligonucleotides KE109A28 and KE110A28 were hybridized to make the 101 bp fragment with StyI and Bam HI staggered ends.

KE109A28: 5' -CAA GGA CGA GTA TGA ACG ACA TAA CAG CTA TAC CTG
TGA GGC CAC TCA CAA GAC ATC AAC TTC ACC CAT TGT CAA GAG CTT
CAA CAG GAA TGA GTG TTA GG- 3' (SEQ ID NO:19)

KE110A28: 5' -GAT CCC TAA CAC TCA TTC CTG TTG AAG CTC TTG ACA
ATG GGT GAA GTT GAT GTC TTG TGA GTG GCC TCA CAG GTA TAG CTG
TTA TGT CGT TCA TAC TCG TC- 3' (SEQ ID NO:20)

Oligonucleotides KE111A28 and KE112A28 were hybridized to make the 71 bp fragment with Xho I and Dde I staggered ends.

KE111A28: 5' -TCG AGG GTA CCG AGC TCT AGA TCT GTA TCC ATC TTC
CCA CCA TCC AGT GAG CAG TTA ACA TCT GGA GGT GCC- 3' (SEQ ID
NO:21)

KE112A28: 5' -TGA GGC ACC TCC AGA TGT TAA CTG CTC ACT GGA TGG
TGG GAA GAT GGA TAC AGA TCT AGA GCT CGG TAC CC- 3' (SEQ ID
NO:22)

Expression vector pCIB4611 contains heavy chain constant regions CH1-CH3, and likewise variable heavy chain regions can be cloned into the Xho I / Bgl II site for expression of a full length heavy chain. The pCIB4611 vector was made by ligating a Nco I /Bst XI 902 bp heavy chain constant region from a mouse IgGG1 Gamma chain (Honjo, *et al.* 1979, Nature 277:627-633; Kabat *et al.*, US Dept Health and Human Services, US Government Printing Offices (1987)) with two 40 bp hybridized oligonucleotide fragments and ligating the final 982 bp fragment into pCIB4610 digested with Bgl II and Xho I. One 40 bp fragment was hybridized from oligos KE106A28 and KE107A28 and has Xho I/Nco I staggered ends and the other 40 bp fragment was hybridized from KE108A28 and KE105A28 and has Bst XI /Bam HI staggered ends.

KE106A28: 5' -TCG AGG GTA CCG AGC TCT AGA TCT GCT GCC CAA ACT
AAC TC- 3' (SEQ ID NO:23)

KE107A28: 5' -CAT GGA GTT AGT TTG GGC AGC AGA TCT AGA GCT CGG
TAC CC- 3' (SEQ ID NO:24)

KE108A28: 5' -CTG GTA AAG GCG GCC GCA TCG ATT AAG TCG ACC CGC
GGG- 3' (SEQ ID NO:25)

KE105A28: 5' -GAT CCC CGC GGG TCG ACT TAA TCG ATG CGG CCG CCT
TTA CCA GGA GA- 3' (SEQ ID NO:26)

The pCIB4610 vector contains a 19 amino acid mouse endoplasmic reticulum signal peptide sequence between CaMV 35S promoter and CaMV 35S termination sequences. The pCIB4610 vector was made by ligating pCIB4600 digested with Bam HI and Hpa I to a 83 bp PCR generated fragment digested with Bam HI and Hpa I. The PCR generated fragment was made using pCIB4600 as a template and PCR primers KE102A28 and KE101A28. PCIB4610 differs from pCIB4600 only in the untranslated

leader region following the CaMV 35S promoter. pCIB4610 contains a plant consensus translational initiation sequence AACAA ATG (SEQ ID NO:27) where ATG is the start of translation, and pCIB4600 contains the sequence TCCG ATG (SEQ ID NO:28).

KE102A28: 5' -CGA AGT TAA CAG ATC TAG AGC TCG G- 3'
(SEQ ID NO: 29)

KE101A28: 5' -CGG GAT CCA ACA ATG GGA TGG AGC TGG ATC TT- 3'
(SEQ ID NO:30)

The pCIB4600 vector was made by ligating a derivative of the CaMV 35S expression vector pCIB710 (Rothstein, *et al.* (1987) Gene 53:153-161) digested with Bam HI and Sac I with a 86 bp Bam HI and Sac I fragment encoding an endoplasmic reticulum signal peptide (Kabat *et al.*, US Dept Health and Human Services, US Government Printing Offices (1987)). The 86 bp fragment contains the following sequence:

5' -GAT CCA ACA ATG GGA TGG AGC TGG ATC TTT CTC TTC CTC CTG TCA
GTT GTT ACC CTA CCT CGA CCT AGA AAG AGA AGG AGG ACA GTG GAG
CTG CAG GTG TCC ATT GCC TAC TCG AGG GTA CCG AGC TCC TCG ACG
TCC ACA GGT AAC GGA TGA GCT CCG ATG GC- 3' (SEQ ID NO:31)

Variable light and heavy chain regions were cloned from five CRW monoclonal lines into the expression vectors to generate the following constructs:

pCIB4613: 3B1 heavy chain variable region

pCIB4614: 3B1 light chain variable region

pCIB4615: 2B5 heavy chain variable region (NRRL B-21216)

pCIB4616: 2B5 light chain variable region (NRRL B-21217)

pCIB4609: 17F6 heavy chain variable region (NRRL B-21215)

pCIB4617: 17F6 light chain variable region (NRRL B-21218)

pCIB4637: 10B6 heavy chain variable region (NRRL B-21279)

pCIB4625: 10B6 light chain variable region (NRRL B-21219)

pCIB4635: 14G1 heavy chain variable region (NRRL B-21277)

pCIB4636: 14G1 light chain variable region (NRRL B-21278)

pCIB4631: 3B1 light and heavy chain variable region (NRRL B-21220)

The expression vectors listed above that are followed by an NRRL accession number were deposited on March 7, 1994 with Agricultural Research Service, Patent Culture Collection (NRRL), Northern Regional Research Center, 1815 North University Street, Peoria, Illinois 61604, U.S.A., with the exception of pCIB4637, pCIB4635 and pCIB4636, which were deposited on June 3, 1994

Table 3 contains a listing of the sequence ID numbers for the variable region sequences. The sequence in the cases of pCIB4613, pCIB4617, pCIB4625, pCIB4637, pCIB4635 and pCIB4636 are complete variable regions starting at the first codon of the first framework region and ending with the last codon of the fourth framework region of the variable region. The variable region in pCIB4609 is not complete, the 5' end of the coding sequence is truncated and the sequence begins in the second CDR region of the variable region.

TABLE 3
LIST OF ANTIBODY CHAIN DNA SEQUENCES

SEQ ID NO:1	3B1 Heavy chain variable region DNA
SEQ ID NO:2	3B1 Heavy chain variable region protein
SEQ ID NO:3	3B1 Light chain variable region DNA
SEQ ID NO:4	3B1 Light chain variable region protein
SEQ ID NO:5	2B5 Heavy chain variable region DNA
SEQ ID NO:6	2B5 Heavy chain variable region protein
SEQ ID NO:7	2B5 Light chain variable region DNA
SEQ ID NO:8	2B5 Light chain variable region protein
SEQ ID NO:9	17F6 Heavy chain variable region DNA
SEQ ID NO:10	17F6 Heavy chain variable region protein
SEQ ID NO:11	17F6 Light chain variable region DNA
SEQ ID NO:12	17F6 Light chain variable region protein
SEQ ID NO:13	10B6 Heavy chain variable region DNA
SEQ ID NO:14	10B6 Heavy chain variable region protein
SEQ ID NO:15	10B6 Light chain variable region DNA
SEQ ID NO:16	10B6 Light chain variable region protein
SEQ ID NO:17	3B1 single chain antibody DNA
SEQ ID NO:18	3B1 single chain antibody protein
SEQ ID NO:44	14G1 Heavy chain variable region DNA
SEQ ID NO:45	14G1 Heavy chain variable region protein
SEQ ID NO:46	14G1 Light chain variable region DNA
SEQ ID NO:47	14G1 Light chain variable region protein

4.2. SYNTHESIS OF DNA OLIGOMERS:

DNA oligomers were synthesized using an Applied Biosystems model 380B DNA synthesizer and standard procedures. The oligomers were made using the updated SSCAF3 cycle on a 0.2 μ mole, wide pore, small scale ABI column. The end procedure was run trityl off and the oligomer was cleaved from the column using the 380B's automatic cleavage cycle. The oligomers were then deblocked in excess ammonium hydroxide (NH_4OH) at 55 C for 8-12 hours. The oligomers were then dried in an evaporator using nitrogen gas. After completion, the oligomers were resuspended in 0.25 - 0.5 ml of deionized water.

4.3. PURIFICATION OF SYNTHETIC DNA OLIGOMERS:

An aliquot of each oligomer was mixed with an equal volume of blue dye/formamide mix with the final solution containing 0.05% bromophenol blue, 0.05% xylene cyanol FF, and 25 % formamide. This mixture was heated at 95 C for 10 minutes to denature the oligomers. Samples were then applied to a 12 % polyacrylamide-urea gel containing 7 M urea (Sambrook *et al.*). After electrophoresis at 300-400 volts for 3-4 hours using a Vertical Slab Gel Unit (Hoefer Scientific Instruments, San Francisco, CA), UV shadowing was used to locate the correct sized fragment in the gel which was then excised using a razor blade. The purified gel fragment was minced and incubated in 0.4 M LiCl, 1 mM EDTA (pH 8) buffer overnight at 37 C.

Either of two methods was then used to separate the oligomers from the polyacrylamide gel remnants: Gel/X (Genex Corp., Gaithersburg) micron porous polyethylene filter units or Millipore's ultrafree-MC 0.45 micron filter units. The purified oligomers were ethanol precipitated, recovered by centrifuging in a microfuge for 20 min at 4 C, and finally resuspended in TE (10 mM Tris, 1 mM EDTA, pH 8.0). Concentrations were adjusted to 50 ng/ μ l based on absorption readings at 260 nm.

4.4. KINASING OLIGOMERS:

In each 20 μ l kinase reaction, one picomole of purified oligomer was used in a buffer of 7.0 mM Tris pH 7.5, 10 mM KCl, 1 mM MgCl_2 , 0.5 mM DTT, 50 μ g/ml BSA, 3000 μ Ci (3 picomoles) of ^{32}P - γ -ATP, and 8 units of T4 polynucleotide kinase. The kinase reaction was incubated for 1 hour at 37 C, followed by a phenol/chloroform

extraction and three ethanol precipitations with glycogen as carrier (Tracy, Prep. Biochem. 11:251-268 (1981)).

4.5..HYBRIDIZING OLIGOMERS FOR DIRECT CLONING:

Oligomers to be hybridized were pooled together (from 1 µg to 20 µg total DNA) and kinased at 37 C for 1 hour in 1X Promega ligation buffer containing 30 mM Tris-HCl pH 7.8, 10 mM MgCl₂, 10 mM DTT, and 1 mM dATP. One to 20 units of T4 polynucleotide kinase was used in the reaction, depending on the amount of total DNA present. The kinasing reactions were stopped by placing the reaction in a boiling water bath for five minutes. The pooled oligomers were in a volume of 50-100 µl with added hybridization buffer used to adjust the final salt conditions to 100 mM NaCl, 120 mM Tris pH 7.5, and 10 mM MgCl₂. The kinased and non-kinased oligomers were pooled together and heated in a boiling water bath for five minutes and allowed to slowly cool to room temperature over a period of about four hours. The hybridized oligomers were then phenol/chloroform extracted, ethanol precipitated, and resuspended in 17 l of TE (10 mM Tris, 1 mM EDTA, pH 8.0). Using this 17 l, a ligation reaction with a final volume of 20 l is assembled (final conditions = 30 mM Tris-HCl pH 7.8, 10 mM MgCl₂, 10 mM DTT, 1 mM ATP, and 3 units of T4 DNA ligase (Promega, Madison WI). The ligation was allowed to incubate for about 2 hours at room temperature. The hybridized/ligated fragments were generally purified on 2% Nusieve agarose (FMC BioProducts, Rockland, ME) gels before and/or after cutting with restriction enzymes and prior to cloning into vectors. A 20 l volume ligation reaction is assembled using 100 ng to 500 ng of each fragment with approximate equimolar amounts of DNA in 30 mM Tris-HCl pH 7.8, 10 mM MgCl₂, 10 mM DTT, 1 mM ATP, and 3 units of T4 DNA ligase (Promega; Madison, WI). Ligations were incubated at room temperature for 2 hours. After ligation, DNA was transformed into frozen competent *E. coli* cells using standard procedures (Sambrook *et al.*) and transformants were selected on LB-agar (Sambrook *et al.*) containing 100 µg/ml ampicillin (see below).

EXAMPLE 5: CONSTRUCTION OF A SINGLE CHAIN ANTIBODY (SCA) MOLECULE

pCIB4631 contains a single chain antibody (SCA) specific to CRW BBMV's fused to the constant regions of the antibody heavy chain gene. The SCA gene contains the fusion of variable fragments from antibody 3B1 light and heavy chains, (from monoclonal antibody line 3B1 specific to CRW BBMV) with a 19 amino acid endoplasmic reticulum signal sequence. Between the light and heavy Fv fragments is a 10 amino acid (GGGGSGGGGS; SEQ ID NO:32) domain linker (Huston *et al.*, PNAS 85:5879-5883 (1988)). pCIB4631 was made by ligating a 4.1 Kb Xba I / Xho I fragment (Fv: constant heavy chain: CaMV 35S termination region: vector fragment from pCIB4613) and a 1.4 Kb Xba I / Bgl II fragment (CaMV 35S promoter: light Fv fragment from pCIB4614), and a hybridized 36 base pair linker fragment with Bgl II / Xho I staggered restriction enzyme site ends.

Oligos KE147A28 and KE182A28 were hybridized together in making a 36 base pair linker:

KE147A28: 5' -GAT CTG GTG GCG GTG GCT CGG GCG GTG GTG GGT
CGC- 3' (SEQ ID NO:33)

KE182A28: 5'-TCG AGC GAC CCA CCA CCG CCC GAG CCA CCG CCA CCA-
3' (SEQ ID NO:34)

Oligomers were purified as described above on a 12 % polyacrylamide/7M urea gel using UV shadowing to cut-out the correct size oligomers using standard procedures. Oligos were kinased and hybridized as described above.

Expression vector pCIB4631 was deposited on March 7, 1994 with the Agricultural Research Service, Patent Culture Collection (NRRL), Northern Regional Research Center, 1815 North University Street, Peoria, Illinois 61604, U.S.A. and was assigned accession number NRRL B-21220.

EXAMPLE 6: CHARACTERIZATION OF SCA BINDING PROPERTIES

Single chain antibody proteins were expressed in maize protoplasts, isolated, and shown to bind CRW BBMV proteins on both a western blot and isolated cross-sections of

CRW midguts in immunosections (Bravo *et al.* 1992, J. of Invert. Path. 60:237-246, Bravo *et al.* 1992, J. of Invert. Path. 60:247-253).

6.1. ISOLATION OF MAIZE SUSPENSION CELL PROTOPLASTS:

Embryogenic suspension cultures derived from immature embryo cultures of a Ciba Seeds maize inbred (B73 type) or alternatively Black Mexican Sweet were maintained in N6 basal medium (Chu *et al.*, 1975) supplemented with 3% sucrose and 2 mg/l 2, 4-D, at 27 C on a gyratory shaker at 130 rpm and sub-cultured weekly. Suspension cells were collected 1-2 days after subculturing and resuspended in enzyme solution (3% cellulase RS + 1% macerozyme R10 dissolved in KMC: 8.7 g/l KCl, 12.5 g/l CaCl₂, 16.4 g/l MgSO₄, 5 g/l MES, pH 5.7) at a ratio of 2 ml packed cell volume per 20 ml enzyme solution. Cells were aliquoted into 100 x 25 mm Petri dishes and incubated for four hours at room temperature on a gyratory shaker at 50 rpm.

6.2. TRANSFORMATION OF PROTOPLASTS:

Immediately after isolation, protoplasts were resuspended at a density of 6 million/ml in RS buffer (0.45 M mannitol, 15 mM CaCl₂, 0.1% MES, pH 5.7). One-half ml aliquots were placed in 17 x 100 mm polystyrene tubes, followed by 50 µg pCIB4631 DNA and 50 µg CaMV 35S GUS such as pB1221 from Clontech Laboratories, Inc., Palo Alto, CA, as transformational control. One-half ml of PEG solution (40% PEG 6000, 0.4 M mannitol, 0.1 M Ca(NO₃)₂) was added to each tube and mixed with protoplasts by gentle shaking. After a 30 min incubation at room temperature, protoplasts were diluted stepwise at 5 minute intervals with 1 ml, 2 ml, 5 ml, and 10 ml W5 (9.0 g/l NaCl, 18.5 g/l CaCl₂, 0.37 g/l KCl, 0.9 g/l glucose pH 5.6), sedimented, and resuspended in plating medium (MS salts, B5 vitamins, 3% sucrose, 2 mg/l 2,4-D, 0.3M mannitol) at a density of 2×10^6 protoplasts/ml. Protoplasts were incubated in the dark at 26 C. At 18-22 hrs, protoplasts were collected in Eppendorf tubes, sedimented, and resuspended in 0.4 ml extraction buffer (100 mM KHPO₄ pH 7.8, 1 mM DTT). Samples were then sonicated for 10 seconds and debris pelleted by centrifugation.

6.3. SINGLE CHAIN ANTIBODY BINDING TO CRW BBMV:

Brush border membrane vesicles were prepared as described above and electrophoresed on 8-16 % acrylamide SDS protein gels (Novex, San Diego, CA).

Proteins were transferred onto nitrocellulose (Burnette, W.N. , Western Blotting, 112:195 (1981) and allowed to bind maize protoplast extracts containing the single chain antibody protein. The CRW BBMV-specific 3B1 single chain antibody protein expressed from pCIB4631 bound the same molecular weight BBMV protein on the western blot as did the original 3B1 monoclonal. The single chain antibody expressed in maize protoplasts was also shown to bind to cross-sections of CRW midgut in immunosectioning experiments (Bravo *et al.* 1992, J. Invert. Path. 60:237-246, Bravo *et al.* 1992, J. Invert. Path. 60:247-253).

EXAMPLE 7: CONSTRUCTION OF CRW SPECIFIC IMMUNOTOXIN

A CRW BBMV specific single chain antibody was fused to the toxic domain from *Pseudomonas* exotoxin A. *Pseudomonas* exotoxin A has been used to synthesize recombinant hybrid antibody-toxin fusion proteins for treatment of cancer and immunological diseases (Pastan, I. and FitzGerald, D., 1989, J. Biol. Chem. 264:15157-15160, and Pastan, I *et al.* Annu. Rev. Biochem. 1992 61:331-54). The structure of *Pseudomonas* exotoxin is well characterized and its mode of action known. The idea of antibody hybrid toxins as insecticidal agents is novel and there is no precedent for this type of approach.

Pseudomonas exotoxin (PE) is a single chain toxin secreted by *Pseudomonas aeruginosa*. It kills cells by catalyzing the irreversible ADP-ribosylation and inactivation of translational elongation factor 2 (EF-2). The structure of PE is well characterized (Chaudhary, V.K. *et al.*, 1990, J. Biol. Chem. 265:16303-16310), and consists of three domains. Domain Ia is responsible for the cell recognition and binding of PE to target cells, domain II is required for the translocation of the ADP-ribosylating activity into the cytosol and domain III is the ADP-ribosylating activity. When the toxin enters the cell it is internalized by endocytic vesicles where cleavage occurs to generate a 37 kD domain III "activated toxin". Deletion of domain Ia removes the cell binding domain and generates a 40 kDa protein (PE40) with "extremely low" cellular cytotoxicity. The fusion of antibodies to PE40 has been used to make many recombinant immunotoxins for cancer therapy. It is believed that the binding of the antibody-PE40 fusion must be followed by internalization by receptor-mediated endocytosis for proper activation of the PE40 and subsequent passage to the cytosol.

The PE toxic domain was fused to the -COOH terminus of the heavy chain fragment of the CRW 3B1 single chain since it has been shown that fusions to the -NH₂ terminus of PE40 retain cytotoxicity. It is also possible to design the fusions such that the single-chain antibody is fused to the -COOH terminus of PE40 (Prior *et al.* Cell Vol. 64:1017-1023). Single-chain antibody fusions were made and tested in *E. coli* expression vectors, using the p-FLAG expression vector which has an IPTG inducible tac promoter followed by sequences encoding the ompA signal peptide for secretion into the periplasm and the eight amino acid FLAG epitope which allows the isolation of recombinant protein by antibody affinity chromatography. Single chain antibody fusion proteins were purified from the FLAG expression vector. The purified SCA fusion proteins are incorporated into a corn rootworm insect diet for activity assays.

A single chain antibody fused to PE40 was made by ligating a ~1.2 Kb Sph I /Eco RI fragment containing PE40 and a 790 bp Hind III / Sph I fragment containing the 3B1 single chain antibody into the 5.37 Kb Hind III / Eco RI digested pFLAG vector (IBI, New Haven, CT). The PE40 fragment was obtained from pWW20, a vector containing the toxic domain of *Pseudomonas* exotoxin A under control of an inducible Lac promoter in a pUC9 vector (Wels *et al.* 1992, Cancer Research 52:6310-6317). The 700 bp fragment containing the 3B1 single chain antibody was generated by PCR using pCIB4631 as template and PCR oligos NC200 and NC202.

NC200: 5' -CGA AGC TTG ACA TTG TGC TGA CCC AG- 3'
(SEQ ID NO:35)

NC202: 5' -GCC CTC TAG AAG CAT GCC TGA GGA GAC GGT GAC TGA- 3'
(SEQ ID NO:36)

EXAMPLE 8: TRANSFORMATION AND EXPRESSION IN PLANTS

Hybrid toxins comprised of antibody domains fused to toxin domains are transformed into plants using current methodology as set forth in WO 93/07278, 08/008,006, and 08/037,057. Binary toxins comprised of two independent antibody chains or antibody domains fused to toxins are expressed and assembled in plants using normal cellular processing. Single chain antibody-toxin proteins are either expressed in the plant cytoplasm,

targeted to the plant apoplast, or in the case of hybrid toxins that have cellular toxicity, targeted to organelles within the plant cell (Taviadoraki *et al.* 1993, *Nature* 366:469-472; Owen *et al.* 1992, *Bio/Technology* 10: 790-794; Firek *et al.* 1993, *Plant Molecular Biology* 23: 861-870). Techniques known in the literature are used to target proteins to the chloroplast or the vacuole via the endoplasmic reticulum. Vacuolar targeting signals in the form of carboxyl-terminal propeptides are described in the literature (Bednarek S. *et al.* 1991, *Plant Cell* 3:1195-1206; Neuhaus J-M *et al.* 1991, *PNAS* 88:10362-10366; Bednarek S. *et al.* 1992, *Plant Mol. Biol.* 20:133-150; Chrispeels M.J. *et al.* 1992, *Cell* 68:613-616; Nakamura K. *et al.* 1993, *Plant Physiol.* 101:1-5; Dombrowski J.E. *et al.* 1993, *Plant Cell* 5:587-596; Schroeder M.R. *et al.* 1993, *Plant Physiol.* 101:451-458). Chloroplast targeting signals in the form of N-terminal transit peptides are described in the literature (Van Den Broeck G. *et al.* 1985, *Nature* 313:358-363; Smeekens S. *et al.* 1987, *Plant Mol. Biol.* 9:377-388; Szabo L.J. *et al.* 1987, In Plant DNA Infectious Agents, eds. T. Hohn and J. Schell, Springer Verlag, Wein, New York, pp. 321-339; Keegstra K. *et al.* 1989, *Annu. Rev. Plant Physiol. Plant Mol. Biol.* 40:471-501).

The present invention provides material and methods for the construction of toxin molecules which are targeted to a particular insect. Insects which have evaded toxin binding and cytotoxic effects of Bt endotoxins are specifically targeted. Furthermore, the toxin molecules are constructed so that they are specific to the particular insect pest.

All publications and patent applications mentioned in this specification are indicative of the level of skill of those skilled in the art to which this invention pertains. All publications and patent applications are herein incorporated by reference to the same extent as if each individual publication or patent application was specifically and individually indicated to be incorporated by reference. Although the foregoing invention has been described in some detail by way of illustration and example for purposes of clarity of understanding, it will be obvious that certain changes and modifications may be practiced within the scope of the claims.

EXAMPLE 9: Formulation Examples

The active ingredient used in following formulation examples are the purified SCA fusion proteins prepared according to Example 7

A1. Wettable powders

	a)	b)	c)
Active ingredient	25%	50%	75%
sodium lignosulfonate	5%	5%	--
sodium laurylsulfate	3%	--	5%
sodium diisobutyl-naphthalenesulfonate	--	6%	10%
octylphenol polyethylene glycol ether (7-8 moles of ethylene oxid)	--	2%	--
highly dispersed silicid acid	5%	10%	10%
kaolin	62%	27%	--

The spores are thoroughly mixed with the adjuvants and the mixture is thoroughly ground in a suitable mill, affording wettable powders which can be diluted with water to give suspensions of the desired concentrations.

A2. Emulsifiable concentrate

Active ingredient	10%
octylphenol polyethylene glycol ether (4-5 moles ethylene oxide)	3%
clacium dodecylbenzensulfonate	3%
castor oil polyglycol ether (36 moles of ethylene oxide)	4%
cyclohexanone	30%
xylene mixture	50%

Emulsions of any required concentration can be obtained from this concentrate by dilution with water.

A3. Dusts

- 47 -

	a)	b)
Active ingredient	5%	8%
talcum	95%	--
kaolin	--	92%

Ready for use dusts are obtained by mixing the active ingredient with the carriers and grinding the mixture in a suitable mill.

A4. Extruder Granulate

Active ingredient	10%
sodium lignosulfonate	2%
carboxymethylcellulose	1%
kaolin	87%

The active ingredient or combination is mixed and ground with the adjuvants and the mixture is subsequently moistened with water. The mixture is extruded, granulated and the dried in a stream of air.

A5. Coated Granule

Active ingredient	3%
polyethylene glycol (mol wt 200)	3%
kaolin	94%

The active ingredient or combination is uniformly applied in a mixer to the kaolin moistened with polyethylene glycol. Non-dusty coated granulates are obtained in this manner.

A6. Suspension Concentrate

Active ingredient	40%
ethylene glycol	10%
nonylphenol polyethylene glycol ether (15 moles of ethylene oxide)	6%
sodium lignosulfonate	10%

- 48 -

carboxymethylcellulose	1%
37% aqueous formaldehyde solution	0.2%
silicone oil in the form of a 75% aqueous solution	0.8%
water	32%

The active ingredient or combination is intimately mixed with the adjuvants giving a suspension concentrate from which suspensions of any desired concentration can be obtained by dilution with water.

BRIEF DESCRIPTION OF THE FIGURE

Figure 1: Western blot analysis of binding of monoclonal antibodies to corn rootworm brush border membrane vesicles after electrophoresis on acrylamide gels. Antibodies from cell lines 3B1, 2B5, 17F6 and 10B6 were used in the analysis. MW = Molecular weight standards.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT:

- (A) NAME: CIBA-GEIGY AG
- (B) STREET: Klybeckstr. 141
- (C) CITY: Basel
- (E) COUNTRY: Switzerland
- (F) POSTAL CODE (ZIP): 4002
- (G) TELEPHONE: +41 61 69 11 11
- (H) TELEFAX: + 41 61 696 79 76
- (I) TELEX: 962 991

(ii) TITLE OF INVENTION: Antibodies which Bind to Insect Gut
Proteins and their Use

(iii) NUMBER OF SEQUENCES: 49

(iv) COMPUTER READABLE FORM:

- (A) MEDIUM TYPE: Floppy disk
- (B) COMPUTER: IBM PC compatible
- (C) OPERATING SYSTEM: PC-DOS/MS-DOS
- (D) SOFTWARE: PatentIn Release #1.0, Version #1.25 (EPO)

(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 357 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(ix) **FEATURE:**

(A) NAME/KEY: CDS

(B) LOCATION: 1..357

(D) OTHER INFORMATION: /note= "3B1 heavy chain variable region from pCIB4613"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

CAG GTC AAA CTG CAG GAG TCT GGT GGA GGA TTG GTG CAG CCT AAA GGG 48
Gln Val Lys Leu Gln Glu Ser Gly Gly Gly Leu Val Gln Pro Lys Gly
1 5 10 15

TCA TTG AAA CTC TCA TGT GCA GCC TCT GGA TTC ACC TTC AAT AAC TTC 96
Ser Leu Lys Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Asn Asn Phe
20 25 30

GCC ATG AAC TGG GTC CGC CAG GCT CCA GGA AAG GGT TTG GAA TGG GTT 144
Ala Met Asn Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
35 40 45

GCT CGC ATA AGA AGT AAA AGT AAT AAT TAT GCA ACA TCT TAT GGC GAT 192
Ala Arg Ile Arg Ser Lys Ser Asn Asn Tyr Ala Thr Ser Tyr Gly Asp
50 55 60

TCA GTG AAA GAC AGG TTC ACC GTC TCC AGA GAT GAT TCA CAA AGC ATG 240
Ser Val Lys Asp Arg Phe Thr Val Ser Arg Asp Asp Ser Gln Ser Met
65 70 75 80

TTC TAT CTG CAA ATG AAC AAC TTG AAA ACT GAG GAC ACA GCC ATG TAT 288
Phe Tyr Leu Gln Met Asn Asn Leu Lys Thr Glu Asp Thr Ala Met Tyr
85 90 95

- 51 -

TAC TGT GTG AGG GTA GTA TAC GGT GCT ATG GAC TAC TGG GGT CAA GGA 336
 Tyr Cys Val Arg Val Val Tyr Gly Ala M t Asp Tyr Trp Gly Gln Gly
 100 105 110

ACC TCA GTC ACC GTC TCC TCA 357
 Thr Ser Val Thr Val Ser Ser
 115

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 119 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

Gln Val Lys Leu Gln Glu Ser Gly Gly Gly Leu Val Gln Pro Lys Gly
 1 5 10 15
 Ser Leu Lys Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Asn Asn Phe
 20 25 30
 Ala Met Asn Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
 35 40 45
 Ala Arg Ile Arg Ser Lys Ser Asn Asn Tyr Ala Thr Ser Tyr Gly Asp
 50 55 60
 Ser Val Lys Asp Arg Phe Thr Val Ser Arg Asp Asp Ser Gln Ser Met
 65 70 75 80
 Phe Tyr Leu Gln Met Asn Asn Leu Lys Thr Glu Asp Thr Ala Met Tyr
 85 90 95

- 52 -

Tyr Cys Val Arg Val Val Tyr Gly Ala Met Asp Tyr Trp Gly Gln Gly
 100 105 110

Thr Ser Val Thr Val Ser Ser
 115

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 333 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:

- (A) NAME/KEY: CDS
- (B) LOCATION: 1..333
- (D) OTHER INFORMATION: /note= "3B1 light chain variable region from pCIB4614 (#21Fv Ab)"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

GAC ATT GTG CTG ACC CAG TCT CCA GCT TCT TTG GCT GTG TCT CTA GGG	48
Asp Ile Val Leu Thr Gln Ser Pro Ala Ser Leu Ala Val Ser Leu Gly	
1 5 10 15	
 CAG AGG GCC ACC ATC TCC TGC AGA GCC AGC GAA AGT GTT GAT CAT TAT	96
Gln Arg Ala Thr Ile Ser Cys Arg Ala Ser Glu Ser Val Asp His Tyr	
20 25 30	
 GAC ATT AGT TTT ATG AAC TGG TTC CAA CAG AAA CCA GGA CAG CCA CCC	144
Asp Ile Ser Phe Met Asn Trp Phe Gln Gln Lys Pro Gly Gln Pro Pro	

- 53 -

35	40	45	
AAA CTC CTC ATC TAT GCT GCA TCC AAC CAA GGA TCC GGG GTC CCT GCC			192
Lys Leu Leu Ile Tyr Ala Ala Ser Asn Gln Gly Ser Gly Val Pro Ala			
50	55	60	
AGG TTT AGT GGC AGT GGG TCT GGG ACA GAC TTC AGC CTC AAC ATC CAT			240
Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Ser Leu Asn Ile His			
65	70	75	80
CCT ATG GAG GAG GAT GAT ACT GCA ATA TAT TTC TGT CAG CAA AGT AGG			288
Pro Met Glu Glu Asp Asp Thr Ala Ile Tyr Phe Cys Gln Gln Ser Arg			
85	90	95	
GAA CTT CCG TAC ACG TTC GGA GGG GGG ACC ACG CTG GAA ATA AAA			333
Glu Leu Pro Tyr Thr Phe Gly Gly Gly Thr Thr Leu Glu Ile Lys			
100	105	110	

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 111 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

Asp	Ile	Val	Leu	Thr	Gln	Ser	Pro	Ala	Ser	Leu	Ala	Val	Ser	Leu	Gly
1				5				10					15		
Gln	Arg	Ala	Thr	Ile	Ser	Cys	Arg	Ala	Ser	Glu	Ser	Val	Asp	His	Tyr
				20				25					30		
Asp	Ile	Ser	Phe	Met	Asn	Trp	Phe	Gln	Gln	Lys	Pro	Gly	Gln	Pro	Pro

- 54 -

35 40 45
 Lys Leu Leu Ile Tyr Ala Ala Ser Asn Gln Gly Ser Gly Val Pro Ala
 50 55 60
 Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Ser Leu Asn Ile His
 65 70 75 80
 Pro Met Glu Glu Asp Asp Thr Ala Ile Tyr Phe Cys Gln Gln Ser Arg
 85 90 95
 Glu Leu Pro Tyr Thr Phe Gly Gly Gly Thr Thr Leu Glu Ile Lys
 100 105 110

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 372 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:

- (A) NAME/KEY: CDS
- (B) LOCATION: 1..372
- (D) OTHER INFORMATION: /note= "2B5 heavy chain variable region from pCIB4615"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

CAG GTG CAA CTG CAG GAG TCT GGA GGA GGC TTG GTA CAG CCT GGG GGT
 Gln Val Gln Leu Gln Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
 1 5 10 15

48

- 55 -

TCT CTG AGA CTC TCC TGT GCA ACT TCT GGG TTC ACC TTC ACT GAT TAC	96
Ser Leu Arg Leu Ser Cys Ala Thr Ser Gly Phe Thr Phe Thr Asp Tyr	
20 25 30	
TAT ATG ACC TGG GTC CGC CAG CCT CCA GGA AAG GCA CTT GAG TGG TTG	144
Tyr Met Thr Trp Val Arg Gln Pro Pro Gly Lys Ala Leu Glu Trp Leu	
35 40 45	
GGT TTT ATT AGA CAC AAA GCT AAT GGT TAC ACA ACA GAA TAC AGT GCA	192
Gly Phe Ile Arg His Lys Ala Asn Gly Tyr Thr Thr Glu Tyr Ser Ala	
50 55 60	
TCT GTG AAG GGT CGG TTC ACC ATC TCC AGA GAT AAT TCC CAA AAC ATC	240
Ser Val Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Gln Asn Ile	
65 70 75 80	
CTC TAT CTT CAA ATG AAC ACC CTG AGA GCT GAG GAC AGT GCC ACT TAT	288
Leu Tyr Leu Gln Met Asn Thr Leu Arg Ala Glu Asp Ser Ala Thr Tyr	
85 90 95	
TAC TGT GCA AGA GAT ATA TGC TAT GGT TAC GAC GTT GGG GCT CTG GAC	336
Tyr Cys Ala Arg Asp Ile Cys Tyr Gly Tyr Asp Val Gly Ala Leu Asp	
100 105 110	
TAC TGG GGT CAA GGA ACC TCA GTC ACC GTC TCC TCA	372
Tyr Trp Gly Gln Gly Thr Ser Val Thr Val Ser Ser	
115 120	

(2) INFORMATION FOR SEQ ID NO:6:

(1) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 124 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

- 56 -

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

Gln Val Gln Leu Gln Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Thr Ser Gly Phe Thr Phe Thr Asp Tyr
20 25 30

Tyr Met Thr Trp Val Arg Gln Pro Pro Gly Lys Ala Leu Glu Trp Leu
35 40 45

Gly Phe Ile Arg His Lys Ala Asn Gly Tyr Thr Thr Glu Tyr Ser Ala
50 55 60

Ser Val Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Gln Asn Ile
65 70 75 80

Leu Tyr Leu Gln Met Asn Thr Leu Arg Ala Glu Asp Ser Ala Thr Tyr
85 90 95

Tyr Cys Ala Arg Asp Ile Cys Tyr Gly Tyr Asp Val Gly Ala Leu Asp
100 105 110

Tyr Trp Gly Gln Gly Thr Ser Val Thr Val Ser Ser
115 120

(2) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 330 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(ix) **FEATURE:**

- (A) NAME/KEY: CDS
(B) LOCATION: 1..330
(D) OTHER INFORMATION: /note= "2B5 light chain variable
region from pCIB4616"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

GAT ATC GTG ATG ACC CAG TCT CCT GCT TCC TTA GCT ATA TCT CTG GGG	48
Asp Ile Val Met Thr Gln Ser Pro Ala Ser Leu Ala Ile Ser Leu Gly	
1 5 10 15	
CAG AGG GCC ACC ATC TCA TAC AGG GCC AGC AAA AGT GTC AGT ACA TCT	96
Gln Arg Ala Thr Ile Ser Tyr Arg Ala Ser Lys Ser Val Ser Thr Ser	
20 25 30	
GGC TAT AGT TAT ATG CAC TGG AAC CAA CAG AAA CCA GGA CAG CCA CCC	144
Gly Tyr Ser Tyr Met His Trp Asn Gln Gln Lys Pro Gly Gln Pro Pro	
35 40 45	
AGA CTC CTC ATC TAT CTT GTA TCC AAC CTA GAA TCT GGG GTC CCT GCC	192
Arg Leu Leu Ile Tyr Leu Val Ser Asn Leu Glu Ser Gly Val Pro Ala	
50 55 60	
AGG TTC AGT GGC AGT GGG TCT GGG ACA GAC TTC ACC CTC AAC ATC CAT	240
Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Asn Ile His	
65 70 75 80	
CCT GTG GAG GAG GAG GAT GCT GCA ACC TAT TAC TGT CAG CAC ATT AGG	288
Pro Val Glu Glu Glu Asp Ala Ala Thr Tyr Tyr Cys Gln His Ile Arg	
85 90 95	
GAG CTT ACA CGT TCG GAG GGG GGA CCA AAG CTG GAA ATA AAA	330
Glu Leu Thr Arg Ser Glu Gly Gly Pro Lys Leu Glu Ile Lys	

- 58 -

100

105

110

(2) INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 110 amino acids

(B) TYPE: amino acid

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

Asp Ile Val Met Thr Gln Ser Pro Ala Ser Leu Ala Ile Ser Leu Gly
 1 5 10 15

Gln Arg Ala Thr Ile Ser Tyr Arg Ala Ser Lys Ser Val Ser Thr Ser
 20 25 30

Gly Tyr Ser Tyr Met His Trp Asn Gln Gln Lys Pro Gly Gln Pro Pro
 35 40 45

Arg Leu Leu Ile Tyr Leu Val Ser Asn Leu Glu Ser Gly Val Pro Ala
 50 55 60

Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Asn Ile His
 65 70 75 80

Pro Val Glu Glu Glu Asp Ala Ala Thr Tyr Tyr Cys Gln His Ile Arg
 85 90 95

Glu Leu Thr Arg Ser Glu Gly Gly Pro Lys Leu Glu Ile Lys
 100 105 110

(2) INFORMATION FOR SEQ ID NO:9:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 165 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(ix) **FEATURE:**

(A) NAME/KEY: CDS

(B) LOCATION: 1..165

(D) OTHER INFORMATION: /note= "17F6 heavy chain variable region"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

TCT GTG AAA GGC AGA TTC ACT ATT TCA AGA GAT GAT TCA CAA AGT ACT 48
Ser Val Lys Gly Arg Phe Thr Ile Ser Arg Asp Asp Ser Gln Ser Thr
1 5 10 15

GTC TAC CTG GAG ATG AAC ACG CTA AGA GAG GAA GAC ACT GCC ACT TAT 96
Val Tyr Leu Glu Met Asn Thr Leu Arg Glu Glu Asp Thr Ala Thr Tyr
20 25 30

TAT TGT TGT AGA GGG GGG GAG GAG GGG TTT CCT TAC TGG GGG CAA GGG 144
Tyr Cys Cys Arg Gly Gly Glu Glu Gly Phe Pro Tyr Trp Gly Gln Gly
35 40 45

ACT CTG GTC ACT GTC TCT GCA 165
Thr Leu Val Thr Val Ser Ala
50 55

(2) INFORMATION FOR SEQ ID NO:10:

- 60 -

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 55 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

Ser Val Lys Gly Arg Phe Thr Ile Ser Arg Asp Asp Ser Gln Ser Thr
1 5 10 15

Val Tyr Leu Glu Met Asn Thr Leu Arg Glu Glu Asp Thr Ala Thr Tyr
20 25 30

Tyr Cys Cys Arg Gly Gly Glu Glu Gly Phe Pro Tyr Trp Gly Gln Gly
35 40 45

Thr Leu Val Thr Val Ser Ala
50 55

(2) INFORMATION FOR SEQ ID NO:11:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 339 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:

- (A) NAME/KEY: CDS
- (B) LOCATION: 1..339
- (D) OTHER INFORMATION: /note= "17F6 light chain variable region"

AAA	339
Lys	

- 62 -

(2) INFORMATION FOR SEQ ID NO:12:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 113 amino acids

(B) TYPE: amino acid

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

Asp Ile Val Leu Thr Gln Ser Pro Ser Ser Leu Ser Val Ser Val Gly
 1 5 10 15

Glu Lys Val Thr Met Ser Cys Lys Ser Ser Gln Ser Leu Phe Asp Ser
 20 25 30

Gly Asn Gln Lys Asn Ser Leu Ala Trp Tyr Gln Gln Lys Pro Gly Gln
 35 40 45

Pro Pro Lys Leu Leu Ile Tyr Gly Thr Ser Thr Arg Asp Ser Gly Val
 50 55 60

Pro Asp Arg Phe Thr Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr
 65 70 75 80

Ile Ser Gly Ile Gln Ala Glu Asp Leu Ala Val Tyr Tyr Cys Gln Asn
 85 90 95

Asp His Tyr Tyr Pro Phe Thr Phe Gly Gly Gly Thr Lys Leu Glu Ile
 100 105 110

Lys

(2) INFORMATION FOR SEQ ID NO:13:

(i) SEQUENCE CHARACTERISTICS:

GCT GTG ATT AAA GTC AAA TCT GCT AAT TAT GGA TCA AAT TAT GCA GAG 192
Ala Val Ile Lys Val Lys Ser Ala Asn Tyr Gly Ser Asn Tyr Ala Glu
50 55 60

- 64 -

TCT GTG AAA GGC AGA TTC ACT ATT TCA AGA GAT GAT TCA AAT AGC GGT 240
 Ser Val Lys Gly Arg Phe Thr Ile Ser Arg Asp Asp Ser Asn Ser Gly
 65 70 75 80

GTC TAC CTG CAG ATG AAC AGA TTA AGA GAA GAA GAC ACT GCC ACT TAT 288
 Val Tyr Leu Gln Met Asn Arg Leu Arg Glu Glu Asp Thr Ala Thr Tyr
 85 90 95

TAT TGT AGT AGA GGG GGG GCC CCC GGG TTT CCT TAT TGG GGC CAA GGG 336
 Tyr Cys Ser Arg Gly Gly Ala Pro Gly Phe Pro Tyr Trp Gly Gln Gly
 100 105 110

ACT CTG GTC ACT GTC TCT GCA 357
 Thr Leu Val Thr Val Ser Ala
 115

(2) INFORMATION FOR SEQ ID NO:14:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 119 amino acids

(B) TYPE: amino acid

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:

Glu Val Lys Val Asp Glu Ser Gly Gly Gly Leu Val Arg Pro Gly Asn
 1 5 10 15

Ser Leu Lys Leu Ser Cys Glu Thr Ser Gly Phe Thr Phe Ser Tyr Tyr
 20 25 30

Trp Met His Trp Leu Arg Gln Pro Pro Gly Lys Arg Leu Glu Trp Ile
 35 40 45

- 65 -

Ala Val Ile Lys Val Lys Ser Ala Asn Tyr Gly Ser Asn Tyr Ala Glu
50 55 60

Ser Val Lys Gly Arg Phe Thr Ile Ser Arg Asp Asp Ser Asn Ser Gly
65 70 75 80

Val Tyr Leu Gln Met Asn Arg Leu Arg Glu Glu Asp Thr Ala Thr Tyr
85 90 95

Tyr Cys Ser Arg Gly Gly Ala Pro Gly Phe Pro Tyr Trp Gly Gln Gly
100 105 110

Thr Leu Val Thr Val Ser Ala
115

(2) INFORMATION FOR SEQ ID NO:15:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 339 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:

- (A) NAME/KEY: CDS
- (B) LOCATION: 1..339
- (D) OTHER INFORMATION: /note= "10B6 light chain variable region"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:15:

- 66 -

GAT ATC GTG ATC ACC CAG TCT CCA TCC TCC CTA AGT GTG TCT TTA GGA	48
Asp Ile Val Ile Thr Gln Ser Pro Ser Ser Leu Ser Val Ser Leu Gly	
1 5 10 15	
GAG AAG GTC ACT TTG AGC TGC AAG TCC AGT CAG AGT CTG TTT ACC GGT	96
Glu Lys Val Thr Leu Ser Cys Lys Ser Ser Gln Ser Leu Phe Thr Gly	
20 25 30	
GGA GAT CAA AAG AAC TCC TTG GCC TGG TAC CAG CAG AAA GCA GGG CAG	144
Gly Asp Gln Lys Asn Ser Leu Ala Trp Tyr Gln Gln Lys Ala Gly Gln	
35 40 45	
CCT CCT AGA CTG TTG ATC TAC GGG ACT TCC ACT AGG GAA TCT GGG GTC	192
Pro Pro Arg Leu Leu Ile Tyr Gly Thr Ser Thr Arg Glu Ser Gly Val	
50 55 60	
CCT GAT CGC TTC ACA GGC AGT GGA TCT GGA ACC GAT TTC ACT CTT GCC	240
Pro Asp Arg Phe Thr Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Ala	
65 70 75 80	
ATC AGC AGT GTG CAG GCT GAA GAC CTG GCA GGT TAT TAC TGT CAG AAT	288
Ile Ser Ser Val Gln Ala Glu Asp Leu Ala Gly Tyr Tyr Cys Gln Asn	
85 90 95	
GAT CAT AGT TAT CCA TTC ACG TTC GGC TCG GGG ACA ATG TTG GAA GTA	336
Asp His Ser Tyr Pro Phe Thr Phe Gly Ser Gly Thr Met Leu Glu Val	
100 105 110	
AAA	339
Lys	

(2) INFORMATION FOR SEQ ID NO:16:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 113 amino acids

- 67 -

(B) TYPE: amino acid

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:16:

Asp Ile Val Ile Thr Gln Ser Pro Ser Ser Leu Ser Val Ser Leu Gly
 1 5 10 15

Glu Lys Val Thr Leu Ser Cys Lys Ser Ser Gln Ser Leu Phe Thr Gly
 20 25 30

Gly Asp Gln Lys Asn Ser Leu Ala Trp Tyr Gln Gln Lys Ala Gly Gln
 35 40 45

Pro Pro Arg Leu Leu Ile Tyr Gly Thr Ser Thr Arg Glu Ser Gly Val
 50 55 60

Pro Asp Arg Phe Thr Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Ala
 65 70 75 80

Ile Ser Ser Val Gln Ala Glu Asp Leu Ala Gly Tyr Tyr Cys Gln Asn
 85 90 95

Asp His Ser Tyr Pro Phe Thr Phe Gly Ser Gly Thr Met Leu Glu Val
 100 105 110

Lys

(2) INFORMATION FOR SEQ ID NO:17:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 1797 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(ix) **FEATURE:**

(A) NAME/KEY: CDS

(B) LOCATION: 1..1797

(D) OTHER INFORMATION: /note= "3B1 single chain antibody
from pCIB4631"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:17:

ATG GGA TGG AGC TGG ATC TTT CTC TTC CTC CTG TCA GGA GCT GCA GGT 48
Met Gly Trp Ser Trp Ile Phe Leu Phe Leu Leu Ser Gly Ala Ala Gly
1 5 10 15

Met Gly Trp Ser Trp Ile Phe Leu Phe Leu Leu Ser Gly Ala Ala Gly
1 5 10 15

1

5

10

15

GTC CAT TGC CTA CTC GAG GAC ATT GTG CTG ACC CAG TCT CCA GCT TCT 96
Val His Cys Leu Leu Glu Asp Ile Val Leu Thr Gln Ser Pro Ala Ser
20 25 30

Val His Cys Leu Leu Glu Asp Ile Val Leu Thr Gln Ser Pro Ala Ser
20 25 30

20

25

30

TTG GCT GTG TCT CTA GGG CAG AGG GCC ACC ATC TCC TGC AGA GCC AGC 144
Leu Ala Val Ser Leu Gly Gln Arg Ala Thr Ile Ser Cys Arg Ala Ser
35 40 45

Leu Ala Val Ser Leu Gly Gln Arg Ala Thr Ile Ser Cys Arg Ala Ser
35 40 45

35

40

45

GAA AGT GTT GAT CAT TAT GAC ATT AGT TTT ATG AAC TGG TTC CAA CAG 192
Glu Ser Val Asp His Tyr Asp Ile Ser Phe Met Asn Trp Phe Gln Gln
50 55 60

Glu Ser Val Asp His Tyr Asp Ile Ser Phe Met Asn Trp Phe Gln Gln
50 55 60

50

55

60

AAA CCA GGA CAG CCA CCC AAA CTC CTC ATC TAT GCT GCA TCC AAC CAA 240
Lys Pro Gly Gln Pro Pro Lys Leu Leu Ile Tyr Ala Ala Ser Asn Gln
65 70 75 80

Lys Pro Gly Gln Pro Pro Lys Leu Leu Ile Tyr Ala Ala Ser Asn Gln
65 70 75 80

65

70

75

80

GGA TCC GGG GTC CCT GCC AGG TTT AGT GGC AGT GGG TCT GGG ACA GAC 288
Gly Ser Gly Val Pro Ala Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp
85 90 95

Gly Ser Gly Val Pro Ala Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp
85 90 95

85

90

95

- 69 -

TTC AGC CTC AAC ATC CAT CCT ATG GAG GAG GAT GAT ACT GCA ATA TAT	336
Phe Ser Leu Asn Ile His Pro Met Glu Glu Asp Asp Thr Ala Ile Tyr	
100 105 110	
TTC TGT CAG CAA AGT AGG GAA CTT CCG TAC ACG TTC GGA GGG GGG ACC	384
Phe Cys Gln Gln Ser Arg Glu Leu Pro Tyr Thr Phe Gly Gly Gly Thr	
115 120 125	
ACG CTG GAA ATA AAA CGG GCT GAT GCT GCA CCA ACT AGA TCT GGT GGC	432
Thr Leu Glu Ile Lys Arg Ala Asp Ala Ala Pro Thr Arg Ser Gly Gly	
130 135 140	
GGT GGC TCG GGC GGT GGT GGG TCG CTC GAG CAG GTC AAA CTG CAG GAG	480
Gly Gly Ser Gly Gly Gly Gly Ser Leu Glu Gln Val Lys Leu Gln Glu	
145 150 155 160	
TCT GGT GGA GGA TTG GTG CAG CCT AAA GGG TCA TTG AAA CTC TCA TGT	528
Ser Gly Gly Gly Leu Val Gln Pro Lys Gly Ser Leu Lys Leu Ser Cys	
165 170 175	
GCA GCC TCT GGA TTC ACC TTC AAT AAC TTC GCC ATG AAC TGG GTC CGC	576
Ala Ala Ser Gly Phe Thr Phe Asn Asn Phe Ala Met Asn Trp Val Arg	
180 185 190	
CAG GCT CCA GGA AAG GGT TTG GAA TGG GTT GCT CGC ATA AGA AGT AAA	624
Gln Ala Pro Gly Lys Gly Leu Glu Trp Val Ala Arg Ile Arg Ser Lys	
195 200 205	
AGT AAT AAT TAT GCA ACA TCT TAT GGC GAT TCA GTG AAA GAC AGG TTC	672
Ser Asn Asn Tyr Ala Thr Ser Tyr Gly Asp Ser Val Lys Asp Arg Phe	
210 215 220	
ACC GTC TCC AGA GAT GAT TCA CAA AGC ATG TTC TAT CTG CAA ATG AAC	720
Thr Val Ser Arg Asp Asp Ser Gln Ser Met Phe Tyr Leu Gln Met Asn	
225 230 235 240	

- 70 -

AAC TTG AAA ACT GAG GAC ACA GCC ATG TAT TAC TGT GTG AGG GTA GTA	768
Asn Leu Lys Thr Glu Asp Thr Ala Met Tyr Tyr Cys Val Arg Val Val	
245 250 255	
TAC GGT GCT ATG GAC TAC TGG GGT CAA GGA ACC TCA GTC ACC GTC TCC	816
Tyr Gly Ala Met Asp Tyr Trp Gly Gln Gly Thr Ser Val Thr Val Ser	
260 265 270	
TCA GCC AAA ACG ACA CCC CCA TCT GTC TAT CCA CTG GCC CCT GGA TCT	864
Ser Ala Lys Thr Thr Pro Pro Ser Val Tyr Pro Leu Ala Pro Gly Ser	
275 280 285	
AGA TCT GCT GCC CAA ACT AAC TCC ATG GTG ACC CTG GGA TGC CTG GTC	912
Arg Ser Ala Ala Gln Thr Asn Ser Met Val Thr Leu Gly Cys Leu Val	
290 295 300	
AAG GGC TAT TTC CCT GAG CCA GTG ACA GTG ACC TGG AAC TCT GGA TCC	960
Lys Gly Tyr Phe Pro Glu Pro Val Thr Val Thr Trp Asn Ser Gly Ser	
305 310 315 320	
CTG TCC AGC GGT GTG CAC ACC TTC CCA GCT GTC CTG CAG TCT GAC CTC	1008
Leu Ser Ser Gly Val His Thr Phe Pro Ala Val Leu Gln Ser Asp Leu	
325 330 335	
TAC ACT CTG AGC AGC TCA GTG ACT GTC CCC TCC AGC ACC TGG CCC AGC	1056
Tyr Thr Leu Ser Ser Ser Val Thr Val Pro Ser Ser Thr Trp Pro Ser	
340 345 350	
GAG ACC GTC ACC TGC AAC GTT GCC CAC CCG GCC AGC AGC ACC AAG GTG	1104
Glu Thr Val Thr Cys Asn Val Ala His Pro Ala Ser Ser Thr Lys Val	
355 360 365	
GAC AAG AAA ATT GTG CCC AGG GAT TGT GGT TGT AAG CCT TGC ATA TGT	1152
Asp Lys Lys Ile Val Pro Arg Asp Cys Gly Cys Lys Pro Cys Ile Cys	
370 375 380	
ACA GTC CCA GAA GTA TCA TCT GTC TTC ATC TTC CCC CCA AAG CCC AAG	1200

GAT GTG CTC ACC ATT ACT CTG ACT OCT AAG GTC ACG TGT GTT GTC GTA 1248
Asp Val Leu Thr Ile Thr Leu Thr Pro Lys Val Thr Cys Val Val Val
405 410 415

GAC ATC AGC AAG GAT GAT CCC GAG GTC CAG TTC AGC TGG TTT GTA GAT 1296
Asp Ile Ser Lys Asp Asp Pro Glu Val Gln Phe Ser Trp Phe Val Asp
420 425 430

GAT GTG GAG GTG CAC ACA GCT CAG ACG CAA CCC CGG GAG GAG CAG TTC 1344
Asp Val Glu Val His Thr Ala Gln Thr Gln Pro Arg Glu Glu Gln Phe
435 440 445

AAC AGC ACT TTC CGC TCA GTC AGT GAA CTT CCC ATC ATG CAC CAG GAC 1392
Asn Ser Thr Phe Arg Ser Val Ser Glu Leu Pro Ile Met His Gln Asp
450 455 460

TGG CTC AAT GGC AAG GAG TTC AAA TGC AGG GTC AAC AGT GCA GCT TTC 1440
Trp Leu Asn Gly Lys Glu Phe Lys Cys Arg Val Asn Ser Ala Ala Phe
465 470 475 480

CCT GCC CCC ATC GAG AAA ACC ATC TCC AAA ACC AAA GGC AGA CCG AAG 1488
Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Thr Lys Gly Arg Pro Lys
485 490 495

GCT CCA CAG GTG TAC ACC ATT CCA CCT CCC AAG GAG CAG ATG GCC AAG 1536
Ala Pro Gln Val Tyr Thr Ile Pro Pro Pro Lys Glu Gln Met Ala Lys
500 505 510

GAT AAA GTC AGT CTG ACC TGC ATG ATA ACA GAC TTC TTC CCT GAA GAC 1584
Asp Lys Val Ser Leu Thr Cys Met Ile Thr Asp Phe Phe Pro Glu Asp
515 520 525

ATT ACT GTG GAG TGG CAG TGG AAT GGG CAG CCA GCG GAG AAC TAC AAG 1632
Ile Thr Val Glu Trp Gln Trp Asn Gly Gln Pro Ala Glu Asn Tyr Lys

- 72 -

530	535	540	
AAC ACT CAG CCC ATC ATG AAC ACG AAT GGC TCT TAC TTC GTC TAC AGC			1680
Asn Thr Gln Pro Ile Met Asn Thr Asn Gly Ser Tyr Phe Val Tyr Ser			
545	550	555	560
AAG CTC AAT GTG CAG AAG AGC AAC TGG GAG GCA GGA AAT ACT TTC ACC			1728
Lys Leu Asn Val Gln Lys Ser Asn Trp Glu Ala Gly Asn Thr Phe Thr			
565	570	575	
TGC TCT GTC TTA CAT GAG GGC CTG CAC AAC CAC CAT ACT GAG AAG AGC			1776
Cys Ser Val Leu His Glu Gly Leu His Asn His His Thr Glu Lys Ser			
580	585	590	
CTC TCC CAC TCT CCT GGT AAA			1797
Leu Ser His Ser Pro Gly Lys			
595			

(2) INFORMATION FOR SEQ ID NO:18:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 599 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:18:

Met	Gly	Trp	Ser	Trp	Ile	Phe	Leu	Phe	Leu	Leu	Ser	Gly	Ala	Ala	Gly
1				5					10					15	
Val	His	Cys	Leu	Leu	Glu	Asp	Ile	Val	Leu	Thr	Gln	Ser	Pro	Ala	Ser
			20						25					30	
Leu	Ala	Val	Ser	Leu	Gly	Gln	Arg	Ala	Thr	Ile	Ser	Cys	Arg	Ala	Ser

- 73 -

35	40	45
Glu Ser Val Asp His Tyr Asp Ile Ser Phe Met Asn Trp Phe Gln Gln		
50	55	60
Lys Pro Gly Gln Pro Pro Lys Leu Leu Ile Tyr Ala Ala Ser Asn Gln		
65	70	75 80
Gly Ser Gly Val Pro Ala Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp		
85	90	95
Phe Ser Leu Asn Ile His Pro Met Glu Glu Asp Asp Thr Ala Ile Tyr		
100	105	110
Phe Cys Gln Gln Ser Arg Glu Leu Pro Tyr Thr Phe Gly Gly Gly Thr		
115	120	125
Thr Leu Glu Ile Lys Arg Ala Asp Ala Ala Pro Thr Arg Ser Gly Gly		
130	135	140
Gly Gly Ser Gly Gly Gly Gly Ser Leu Glu Gln Val Lys Leu Gln Glu		
145	150	155 160
Ser Gly Gly Gly Leu Val Gln Pro Lys Gly Ser Leu Lys Leu Ser Cys		
165	170	175
Ala Ala Ser Gly Phe Thr Phe Asn Asn Phe Ala Met Asn Trp Val Arg		
180	185	190
Gln Ala Pro Gly Lys Gly Leu Glu Trp Val Ala Arg Ile Arg Ser Lys		
195	200	205
Ser Asn Asn Tyr Ala Thr Ser Tyr Gly Asp Ser Val Lys Asp Arg Phe		
210	215	220
Thr Val Ser Arg Asp Asp Ser Gln Ser Met Phe Tyr Leu Gln Met Asn		
225	230	235 240

- 74 -

Asn Leu Lys Thr Glu Asp Thr Ala Met Tyr Tyr Cys Val Arg Val Val
 245 250 255

Tyr Gly Ala Met Asp Tyr Trp Gly Gln Gly Thr Ser Val Thr Val Ser
 260 265 270

Ser Ala Lys Thr Thr Pro Pro Ser Val Tyr Pro Leu Ala Pro Gly Ser
 275 280 285

Arg Ser Ala Ala Gln Thr Asn Ser Met Val Thr Leu Gly Cys Leu Val
 290 295 300

Lys Gly Tyr Phe Pro Glu Pro Val Thr Val Thr Trp Asn Ser Gly Ser
 305 310 315 320

Leu Ser Ser Gly Val His Thr Phe Pro Ala Val Leu Gln Ser Asp Leu
 325 330 335

Tyr Thr Leu Ser Ser Ser Val Thr Val Pro Ser Ser Thr Trp Pro Ser
 340 345 350

Glu Thr Val Thr Cys Asn Val Ala His Pro Ala Ser Ser Thr Lys Val
 355 360 365

Asp Lys Lys Ile Val Pro Arg Asp Cys Gly Cys Lys Pro Cys Ile Cys
 370 375 380

Thr Val Pro Glu Val Ser Ser Val Phe Ile Phe Pro Pro Lys Pro Lys
 385 390 395 400

Asp Val Leu Thr Ile Thr Leu Thr Pro Lys Val Thr Cys Val Val Val
 405 410 415

Asp Ile Ser Lys Asp Asp Pro Glu Val Gln Phe Ser Trp Phe Val Asp
 420 425 430

- 75 -

Asp Val Glu Val His Thr Ala Gln Thr Gln Pro Arg Glu Glu Gln Phe
 435 440 445

Asn Ser Thr Phe Arg Ser Val Ser Glu Leu Pro Ile Met His Gln Asp
 450 455 460

Trp Leu Asn Gly Lys Glu Phe Lys Cys Arg Val Asn Ser Ala Ala Phe
 465 470 475 480

Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Thr Lys Gly Arg Pro Lys
 485 490 495

Ala Pro Gln Val Tyr Thr Ile Pro Pro Pro Lys Glu Gln Met Ala Lys
 500 505 510

Asp Lys Val Ser Leu Thr Cys Met Ile Thr Asp Phe Phe Pro Glu Asp
 515 520 525

Ile Thr Val Glu Trp Gln Trp Asn Gly Gln Pro Ala Glu Asn Tyr Lys
 530 535 540

Asn Thr Gln Pro Ile Met Asn Thr Asn Gly Ser Tyr Phe Val Tyr Ser
 545 550 555 560

Lys Leu Asn Val Gln Lys Ser Asn Trp Glu Ala Gly Asn Thr Phe Thr
 565 570 575

Cys Ser Val Leu His Glu Gly Leu His Asn His His Thr Glu Lys Ser
 580 585 590

Leu Ser His Ser Pro Gly Lys
 595

(2) INFORMATION FOR SEQ ID NO:19:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 101 base pairs

- 76 -

- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid

(A) DESCRIPTION: oligonucleotide KE109A28 used to make
101bp Sty I/Bgl II fragment for pCIB4612

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:19:

CAAGGACGAG TATGAACGAC ATAACAGCTA TACCTGTGAG GCCACTCACA AGACATCAAC 60

TTCACCCATT GTCAAGAGCT TCAACAGGAA TGAGTGTAG G 101

(2) INFORMATION FOR SEQ ID NO:20:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 101 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid

(A) DESCRIPTION: oligonucleotide KE110A28 used to make
101bp Sty I/Bgl II fragment for pCIB4612

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:20:

GATCCCTAAC ACTCATTCTT GTTGAAGCTC TTGACAATGG GTGAAGTTGA TGTCTTGTGA 60

GTGGCCTCAC AGGTATAGCT GTTATGTGCT TCATACTCGT C 101

(2) INFORMATION FOR SEQ ID NO:21:

- 77 -

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 72 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid

- (A) DESCRIPTION: oligonucleotide KE111A28 used to make 71bp Xho I/Dde I fragment for pCIB4612

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:21:

TCGAGGGTAC CGAGCTCTAG ATCTGTATCC ATCTTCCAC CATCCAGTGA GCAGTTAACA 60

TCTGGAGGTG CC 72

(2) INFORMATION FOR SEQ ID NO:22:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 71 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid

- (A) DESCRIPTION: oligonucleotide KE112A28 used to make 71bp Xho I/Dde I fragment for pCIB4612

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:22:

TGAGGCACCT CCAGATGTTA ACTGCTCACT GGATGGTGGG AAGATGGATA CAGATCTAGA 60

GCTCGGTACC C 71

- 78 -

(2) INFORMATION FOR SEQ ID NO:23:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 41 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid

- (A) DESCRIPTION: oligonucleotide KE106A28 used to make 40bp Xho I/Nco I fragment for pCIB4611

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:23:

TCGAGGGTAC CGAGCTCTAG ATCTGCTGCC CAAACTAACT C

41

(2) INFORMATION FOR SEQ ID NO:24:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 41 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid

- (A) DESCRIPTION: oligonucleotide KE107A28 used to make 40bp Xho I/Nco I fragment for pCIB4611

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:24:

CATGGAGTTA GTTTGGGCAG CAGATCTAGA GCTCGGTACC C

41

(2) INFORMATION FOR SEQ ID NO:25:

- 79 -

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 39 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid

- (A) DESCRIPTION: oligonucleotide KE108A28 used to make 40bp Bst XI/Bam HI fragment for pCIB4611

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:25:

CTGGTAAAGG CGGCCGCATC GATTAAAGTCG ACCCGCGGG

39

(2) INFORMATION FOR SEQ ID NO:26:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 47 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid

- (A) DESCRIPTION: oligonucleotide KE105A28 used to make 40bp Bst XI/Bam HI fragment for pCIB4611

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:26:

GATCCCCGCG GGTGACTTA ATCGATGCGG CGCCTTTAC CAGGAGA

47

(2) INFORMATION FOR SEQ ID NO:27:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 7 base pairs
- (B) TYPE: nucleic acid

- 80 -

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid

(A) DESCRIPTION: plant consensus translational initiation
sequence for pCIB4610

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:27:

AACAAATG

7

(2) INFORMATION FOR SEQ ID NO:28:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 7 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid

(A) DESCRIPTION: plant consensus translational initiation
sequence for pCIB4600

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:28:

TCCGATG

7

(2) INFORMATION FOR SEQ ID NO:29:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 25 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

- 81 -

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid

(A) DESCRIPTION: PCR primer KE102A28 used to generate 83bp
fragment for pCIB4610

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:29:

CGAAGTTAAC AGATCTAGAG CTCGG

25

(2) INFORMATION FOR SEQ ID NO:30:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 32 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid

(A) DESCRIPTION: PCR primer KE101A28 used to generate 83bp
fragment for pCIB4610

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:30:

CGGGATCCAA CAATGGGATG GAGCTGGATC TT

32

(2) INFORMATION FOR SEQ ID NO:31:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 164 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

- 82 -

(ii) MOLECULE TYPE: other nucleic acid

(A) DESCRIPTION: oligonucleotide encoding an endoplasmic reticulum signal peptide from Kabat et al., 1987

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:31:

GATCCAACAA TGGGATGGAG CTGGATCTTT CTCTTCTCC TGTCAGTTGT TACCCTACCT	60
CGACCTAGAA AGAGAAGGAG GACAGTGGAG CTGCAGGTGT CCATTGCCTA CTCGAGGGTA	120
CCGAGCTCCT CGACGTCCAC AGGTAACGGA TGAGCTCCGA TGGC	164

(2) INFORMATION FOR SEQ ID NO:32:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 10 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(v) FRAGMENT TYPE: internal

(ix) FEATURE:

- (A) NAME/KEY: Domain
- (B) LOCATION: 1..10
- (D) OTHER INFORMATION: /note= "10 amino acid domain linker
between light and heavy Fv fragments"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:32:

- 83 -

Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser
1 5 10

(2) INFORMATION FOR SEQ ID NO:33:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 36 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid

- (A) DESCRIPTION: oligonucleotide KE147A28 used to make 36bp linker for pCIB4631

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:33:

GATCTGGTGG CGGTGGCTCG GGCGGTGGTG GGTCGC

36

(2) INFORMATION FOR SEQ ID NO:34:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 36 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid

- (A) DESCRIPTION: oligonucleotide KE182A28 used to make 36bp linker for pCIB4631

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:34:

TCGAGCGACC CACCACCGCC CGAGCCACCG CCACCA

36

- 84 -

(2) INFORMATION FOR SEQ ID NO:35:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 26 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid

(A) DESCRIPTION: PCR primer NC200 used to generate 700bp fragment containing the 3B1 single chain antibody coding sequence for fusion to PE40

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:35:

CGAAGCTTGA CATTGTGCTG ACCCAG

26

(2) INFORMATION FOR SEQ ID NO:36:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 36 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid

(A) DESCRIPTION: PCR primer NC202 used to generate 700bp fragment containing the 3B1 single chain antibody coding sequence for fusion to PE40

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:36:

GCCCTCTAGA AGCATGCCTG AGGAGACGGT GACTGA

36

- 85 -

(2) INFORMATION FOR SEQ ID NO:37:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 29 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid

- (A) DESCRIPTION: PCR primer NC92 used to amplify antibody genes

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:37:

GTCTCGAGGA YATYSWGMTS ACCCARTCT

29

(2) INFORMATION FOR SEQ ID NO:38:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 29 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid

- (A) DESCRIPTION: PCR primer NC130 used to amplify antibody genes

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:38:

GCAGATCTAG TTGGTGCAGC ATCAGCCCG

29

(2) INFORMATION FOR SEQ ID NO:39:

- 86 -

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 30 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid

- (A) DESCRIPTION: PCR primer NC91 used to amplify antibody genes

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:39:

GTCTCGAGCA GGTSMARCTG CAGSAGTCWG

30

(2) INFORMATION FOR SEQ ID NO:40:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 29 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid

- (A) DESCRIPTION: PCR primer NC114 used to amplify antibody genes

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:40:

GCAGATCTAG ATCCAGGGGC CAGTGGATA

29

(2) INFORMATION FOR SEQ ID NO:41:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 32 base pairs

- 87 -

- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid

- (A) DESCRIPTION: PCR primer NC111 used to amplify antibody genes

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:41:

GCAGATCTGC AGGAGACGAG GGGGAAGACA TT

32

(2) INFORMATION FOR SEQ ID NO:42:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 29 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid

- (A) DESCRIPTION: PCR primer NC117 used to amplify antibody genes

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:42:

GCAGATCTGC AGCCAGGGAC CAAGGGATA

29

(2) INFORMATION FOR SEQ ID NO:43:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 22 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single

- 88 -

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: NO

(v) FRAGMENT TYPE: internal

(ix) FEATURE:

(A) NAME/KEY: Domain

(B) LOCATION: 1..22

(D) OTHER INFORMATION: /note= "alternative domain linker
between light and heavy Fv fragments"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:43:

Gly Pro Gly Pro Ser Thr Pro Pro Thr Pro Ser Pro Ser Thr Pro Pro
1 5 10 15

Thr Pro Ser Gly Pro Gly
 20

(2) INFORMATION FOR SEQ ID NO:44:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 357 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(ix). **FEATURE:**

(A) NAME/KEY: CDS

(B) LOCATION: 1..357

(D) OTHER INFORMATION: /note= "14G1 heavy chain variable region from pCIB4635"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:44:

GAG GTG AAG CTT GTG GAG TCT GGG GGA GGC TTG GTG AGG CCT GGA AAT 48
Glu Val Lys Leu Val Glu Ser Gly Gly Gly Leu Val Arg Pro Gly Asn
1 5 10 15

TCT CTG AAA CTC TCC TGT GTT ACC TCG GGA TTC ACT TTC AGT AAC TAC 96
Ser Leu Lys Leu Ser Cys Val Thr Ser Gly Phe Thr Phe Ser Asn Tyr
20 25 30

CGG ATG CAC TGG CTT CGC CAG CCT CCA GGG AAG AGG CTG GAG TGG ATT 144
Arg Met His Trp Leu Arg Gln Pro Pro Gly Lys Arg Leu Glu Trp Ile
35 40 45

GCT GTA ATT ACA CTC AAA TCT GAT AAT TAT GGA ACA ATT TAT GCA GAA 192
Ala Val Ile Thr Leu Lys Ser Asp Asn Tyr Gly Thr Ile Tyr Ala Glu
50 55 60

TCT GTG AAA GGC AGA TTC ACC ATT TCA AGA GAA GAT TCA GAA AGC AGC 240
Ser Val Lys Gly Arg Phe Thr Ile Ser Arg Glu Asp Ser Glu Ser Ser
65 70 75 80

ATC TAC CTG CAG ATG AAC AGA TTA AGA GAG GAA GAC ACT GCC ACT TAT 288
Ile Tyr Leu Gln Met Asn Arg Leu Arg Glu Glu Asp Thr Ala Thr Tyr
85 90 95

TAC TGT AGT AGA GGT AGT GAC TGG GGA TTT CCT TAT TGG GGG CAA GGG 336
Tyr Cys Ser Arg Gly Ser Asp Trp Gly Phe Pro Tyr Trp Gly Gln Gly

- 90 -

100 105 110

ACT CTG GTC ACT GTC TCT GCA 357

Thr Leu Val Thr Val Ser Ala

115

(2) INFORMATION FOR SEQ ID NO:45:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 119 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:45:

Glu Val Lys Leu Val Glu Ser Gly Gly Gly Leu Val Arg Pro Gly Asn
 1 5 10 15

Ser Leu Lys Leu Ser Cys Val Thr Ser Gly Phe Thr Phe Ser Asn Tyr
 20 25 30

Arg Met His Trp Leu Arg Gln Pro Pro Gly Lys Arg Leu Glu Trp Ile
 35 40 45

Ala Val Ile Thr Leu Lys Ser Asp Asn Tyr Gly Thr Ile Tyr Ala Glu
 50 55 60

Ser Val Lys Gly Arg Phe Thr Ile Ser Arg Glu Asp Ser Glu Ser Ser
 65 70 75 80

Ile Tyr Leu Gln Met Asn Arg Leu Arg Glu Glu Asp Thr Ala Thr Tyr
 85 90 95

Tyr Cys Ser Arg Gly Ser Asp Trp Gly Phe Pro Tyr Trp Gly Gln Gly

- 91 -

100

105

110

Thr Leu Val Thr Val Ser Ala

115

(2) INFORMATION FOR SEQ ID NO:46:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 339 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(ix) FEATURE:

- (A) NAME/KEY: CDS
- (B) LOCATION: 1..339
- (D) OTHER INFORMATION: /note= "14G1 light chain variable region from pCIB4636"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:46:

GAT	ATT	GTG	ATG	ACC	CAG	TCT	CCA	TCC	TCC	CTG	AGT	GTG	TCA	GCA	GGA	48
Asp	Ile	Val	Met	Thr	Gln	Ser	Pro	Ser	Ser	Leu	Ser	Val	Ser	Ala	Gly	
1				5						10					15	
GAG	AAG	GTC	ACT	ATG	AAC	TGC	AAG	TCC	AGT	CAG	AGT	CTG	TTA	AAT	AGT	96
Glu	Lys	Val	Thr	Met	Asn	Cys	Lys	Ser	Ser	Gln	Ser	Leu	Leu	Asn	Ser	
				20						25					30	

- 92 -

GGA AAT CAA AAG CAC TAC TTG GCC TGG TAC CAG CAG AAA CCA GGC CAG	144
Gly Asn Gln Lys His Tyr Leu Ala Trp Tyr Gln Gln Lys Pro Gly Gln	
35 40 45	
 CCT CCT AAA CTG TTG ATC TAC GGG GCA TCC ACT AGG GAA TCT GGG GTC	 192
Pro Pro Lys Leu Leu Ile Tyr Gly Ala Ser Thr Arg Glu Ser Gly Val	
50 55 60	
 CCT GAT CGC TTC ACA GGC AGT GGG TCT GGA ACC GAT TTC ACT CTT ACC	 240
Pro Asp Arg Phe Thr Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr	
65 70 75 80	
 ATC AGC AGT GTG CAG GCT GAA GAC CTG GCA GTT TAT TTC TGT CAG AAT	 288
Ile Ser Ser Val Gln Ala Glu Asp Leu Ala Val Tyr Phe Cys Gln Asn	
85 90 95	
 GAT CGT AGT TAT CCG TTC ACA TTC GCC TCG GGG ACA AAG TTG GAA ATA	 336
Asp Arg Ser Tyr Pro Phe Thr Phe Ala Ser Gly Thr Lys Leu Glu Ile	
100 105 110	
 AAA	 339
Lys	

(2) INFORMATION FOR SEQ ID NO:47:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 113 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:47:

Asp Ile Val Met Thr Gln Ser Pro Ser Ser Leu Ser Val Ser Ala Gly

- 93 -

1	5	10	15
Glu Lys Val Thr Met Asn Cys Lys Ser Ser Gln Ser Leu Leu Asn Ser			
20	25	30	
Gly Asn Gln Lys His Tyr Leu Ala Trp Tyr Gln Gln Lys Pro Gly Gln			
35	40	45	
Pro Pro Lys Leu Leu Ile Tyr Gly Ala Ser Thr Arg Glu Ser Gly Val			
50	55	60	
Pro Asp Arg Phe Thr Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr			
65	70	75	80
Ile Ser Ser Val Gln Ala Glu Asp Leu Ala Val Tyr Phe Cys Gln Asn			
85	90	95	
Asp Arg Ser Tyr Pro Phe Thr Phe Ala Ser Gly Thr Lys Leu Glu Ile			
100	105	110	
Lys			

(2) INFORMATION FOR SEQ ID NO:48:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 32 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid

- (A) DESCRIPTION: PCR primer DB91 used to amplify antibody genes

(iii) HYPOTHETICAL: NO

- 94 -

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:48:

ACGTCTCGAG GARGTGAAGC TKRWKGARWC TG

32

(2) INFORMATION FOR SEQ ID NO:49:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 34 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid

- (A) DESCRIPTION: PCR primer DB114 used to amplify antibody genes

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:49:

CAATTCGCAT ATGAGATCCA GGGGCCAGTG GATA

34

What is claimed is:

1. An antibody or fragment thereof which binds to the gut of a target insect but does not bind to mammalian brush border membranes or to plant microsomes.
2. The antibody of claim 1 wherein said antibody is a monoclonal antibody or binding fragment thereof.
3. The monoclonal antibody or fragment thereof of claim 2 wherein said monoclonal antibody or binding fragment binds to brush border membranes of insects.
4. The monoclonal antibody of claims 1 or 2 wherein said target insect is selected from the orders Coleoptera, Diptera, Hymenoptera, Lepidoptera, Mallophaga, Homoptera, Hemiptera, Orthoptera, Thysanoptera, Dermaptera, Isoptera, Anoplura, Siphonaptera, and Trichoptera.
5. The monoclonal antibody of claim 4 wherein said target insect is western corn root worm.
6. The monoclonal antibody of claim 5 wherein said antibody is selected from the group consisting of 2B5, 3B1, 10B6, 17F6, 14G1 and 16E4.
7. A DNA sequence which encodes the monoclonal antibody or the binding site of said monoclonal antibody of any one of claims 2 to 6.
8. The DNA sequence of claim 7 wherein said DNA sequence is selected from SEQ. ID Nos 1, 3, 5, 7, 9, 11, 13, 15, 17, 44 or 46.
9. The DNA sequence of claims 7 or 8 wherein said DNA sequence is operably linked to a DNA sequence encoding a toxin moiety.

10. The DNA sequence of claim 9 wherein said toxin moiety is selected from *Bacillus* toxins, *Pseudomonas* exotoxin, phytolectin, gelonin, ribonucleases or ribosome inactivating proteins.
11. The DNA sequence of any one of claims 7 to 10, wherein the said DNA is part of a plant genome.
12. A hybrid toxin comprising an antibody or fragment thereof which binds to the gut of a target insect but does not bind to mammalian brush border membranes or to plant microsomes operably linked to a toxin moiety.
13. A hybrid toxin molecule said molecule comprising the monoclonal antibody or monoclonal antibody binding fragment of claim 2 operably linked with a toxin moiety.
14. The hybrid toxin of claims 12 or 13 wherein said toxin moiety is selected from *Bacillus* toxins, *Pseudomonas* exotoxin, phytolectin, gelonin, ribonucleases or ribosome inactivating proteins.
15. The hybrid toxin of claim 14 wherein said *Bacillus* toxin is selected from *Bacillus* endotoxins and vegetative insecticidal proteins.
16. The hybrid toxin of claim 15 wherein said *Bacillus* endotoxin is a *Bt* endotoxin.
17. The hybrid toxin of claim 12, wherein said monoclonal antibody is an antibody according to any one of claims 2 to 6.
18. The hybrid toxin of claim 17 wherein said monoclonal antibody or binding region thereof binds to insect brush border membranes.
19. The hybrid toxin of claim 18 wherein said monoclonal antibody is selected from the group consisting of 2B5, 3B1, 10B6, 17F6, 14G1 and 16E4.
20. A DNA sequence which encodes the hybrid toxin of any one of claims 12-19.

21. A DNA sequence according to claim 20 which sequence is part of the plant genome.
22. A DNA sequence consisting of a first expression cassette comprising a DNA sequence which encodes a promoter capable of directing expression in a plant operably linked to a DNA sequence which encodes a light chain of a monoclonal antibody or antibody binding domain wherein said monoclonal antibody binds the gut of a target insect.
23. The DNA sequence of claim 22 wherein said cassette further comprises a DNA sequence which encodes a toxin moiety operably linked to the DNA sequence which encodes the light chain of the monoclonal antibody or antibody binding domain.
24. A DNA sequence comprising a second expression cassette comprising a DNA sequence encoding a promoter capable of directing expression in a plant operably linked to a DNA sequence which encodes a heavy chain of a monoclonal antibody or antibody binding domain wherein said monoclonal antibody binds the gut of a target insect.
25. The DNA sequence of claim 24 wherein said cassette further comprises a DNA sequence which encodes a toxin moiety operably linked to the DNA sequence which encodes the heavy chain of the monoclonal antibody or antibody binding domain.
26. The DNA sequence of any one of claims 22 to 25 which in addition comprise a termination sequence operable in plants.
27. The DNA sequence of claims 23 or 26 wherein said toxin moiety is selected from *Bacillus* toxins, *Pseudomonas* exotoxin, phytolaccin, or ribonucleases.
28. The DNA sequence of any one of claims 22 to 27 wherein the said DNA is part of plant genome.
29. A plant cell transformed with a DNA sequence of any one of claims 7 to 11 and 20 to 21.
30. A plant cell transformed with a DNA sequence of any one of claims 22 to 28.

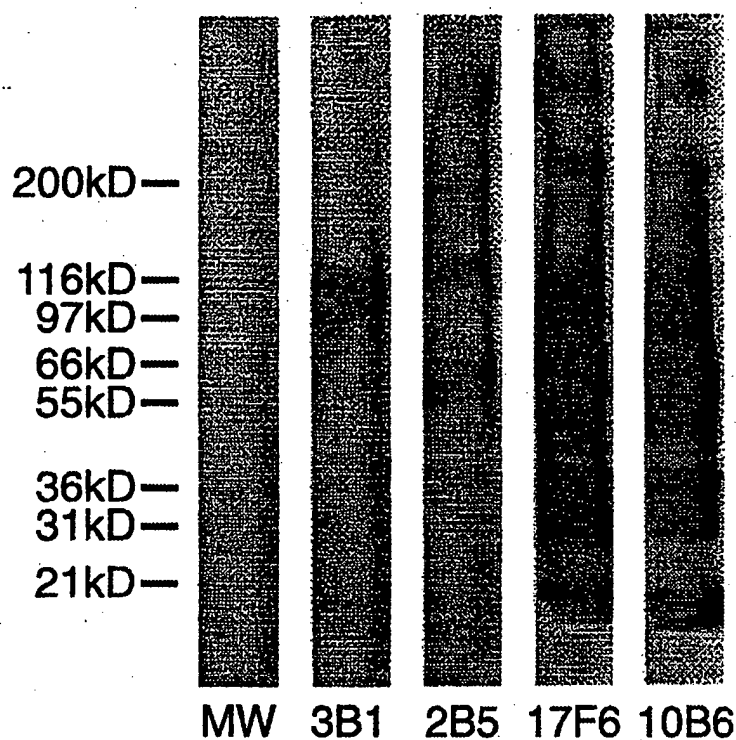
31. A plant and the progeny thereof transformed with a DNA sequence of any one of claims 7 to 11 and 20 to 21.
32. A plant and the progeny thereof transformed with a DNA sequence of any one of claims 22 to 28.
33. A plant and the progeny thereof expressing a monoclonal antibody according to any one of claims 1 to 6.
34. A plant and the progeny thereof expressing a hybrid toxin according to any one of claims 12 to 19.
35. A plant according to any one of claims 31 to 34 which is a maize plant.
36. A plant according to any one of claims 31 to 35 which is a hybrid plant.
37. Propagating material of a plant according to any one of claims 31 to 36 treated with a protectant coating.
38. Propagating material according to claim 37, comprising a preparation selected from the group consisting of herbicides, insecticides, fungicides, bactericides, nematocides, molluscicides or mixtures thereof.
39. Propagating material according to claim 37 or 38 characterized in that it consists of seed.
40. A recombinant microorganism transformed with at least one of the DNA sequences according to any one of claims 7 to 11 and 20 to 21.
41. A recombinant microorganism transformed with at least one of the isolated DNA sequences according to any one of claims 22 to 28.

42. A recombinant microorganism comprising at least one DNA molecule encoding a hybrid toxin comprising an antibody or fragment thereof which binds to the gut of a target insect but does not bind to mammalian brush border membranes or to plant microsomes operably linked to a toxin moiety wherein the said DNA encoding a monoclonal antibody comprises a sequence selected from the group consisting of SEQ. ID Nos 1, 3, 5, 7, 9, 11, 13, 15, 17, 44 or 46.
43. The recombinant microorganism of claims 40-42 wherein said recombinant microorganism is selected from the group consisting of bacteria such as *Bacillus*, *Caulobacter*, *Agmenellum*, *Pseudomonas*, *Erwinia*, *Serratia*, *Klebsiella*, *Xanthomonas*, *Streptomyces*, *Rhizobium*, *Rhodopseudomonas*, *Methylius*, *Agrobacterium*, *Acetobacter*, *Lactobacillus*, *Arthrobacter*, *Azotobacter*, *Leuconostoc*, and *Alcaligenes*; fungi, particularly yeast, such as *Saccharomyces*, *Cryptococcus*, *Kluyveromyces*, *Sporobolomyces*, *Rhodotorula*, and *Aureobasidium* and viruses such as the nuclear polyhedrosis virus *Autographica californica*.
44. An entomocidal composition comprising the recombinant microorganism of any one of claims 40 to 43 in an insecticidally-effective amount together with a suitable carrier.
45. An entomocidal composition comprising an isolated hybrid toxin molecule according to any one of claims 12 to 19 in an insecticidally-effective amount together with a suitable carrier.
46. Propagating material of a plant, which is treated with an entomocidal composition according to any one of claims 44 to 45.
47. Propagating material according to claim 46 characterized in that it is plant seed.
48. A process of the stable transformation of a host organism comprising introducing a DNA sequence according to a sequence of anyone of claims 7 to 10 or 21 or of 23 to 25 or of 27 to 29 into the genome of the said host organism.
49. A process according to claim 33, wherein the host organism is a microorganism.

50. A process according to claim 33., wherein the host organism is a plant.
51. A process for the preparation of a hybridoma cell line which produces an antibody or monoclonal antibody according to claim 1, comprising
- a) utilizing insect guts, particularly insect brush border membranes, as the antigen;
 - b) immunising a donor animal with said antigen;
 - c) isolating an immunocompetent B cell from the immunised donor animal;
 - d) fusing said immunocompetent B cell with a tumour cell line which is capable of continuous cell division;
 - e) isolating the resulting fusion product, cultivating it in a suitable culture medium and subsequently cloning positive hybrid cells; and
 - f) screening the cloned hybrid cells for the production of monoclonal antibodies, and selecting those which show the required properties.
52. A process for the construction of hybrid antibody-toxin molecules according to claim 11-20 comprising cloning the variable region of the antibody and linking said region to the toxin moiety.
53. A process of producing a monoclonal antibody or fragments thereof which bind to brush border membrane vesicles of the insect gut comprising growing the hybridoma cell lines producing the said antibodies in a suitable cultivation medium *in vivo* or *in vitro* and isolating the resulting antibodies.
54. A process of producing a DNA sequence encoding a monoclonal antibody according to claim 1 comprising cloning the respective antibody genes from hybridoma cells using primers to conserved DNA sequences within the constant regions and the framework regions of the variable regions.
55. A hybridoma cell line producing a monoclonal antibody according to any one of claims 2 to 6.
56. A hybridoma cell line according to claim 55 which has been deposited under accession number ATCC HB 11616, HB 11617, HB 11618, HB 11619 and HB 11620.

1/1

Antibody Binding to Corn Rootworm Brush Border Membrane Vesicles



INTERNATIONAL SEARCH REPORT

Inter. Appl. No.
PCT/IB 95/00497

A. CLASSIFICATION OF SUBJECT MATTER
 IPC 6 C12N15/13 C12N15/31 C12N15/62 C12N15/82 C07K16/18
 C07K16/28 C12N5/10 A01H5/00 C12N1/21 C12P21/08
 C12N5/12 A01N63/02

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 6 C12N C12P C07K A01H A01N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	INSECT BIOCHEM., vol. 11, no. 5, 1981 pages 593-598, CRANKSHAW, D.L., ET AL. 'INTERSPECIES CROSS-REACTIVITY OF AN ANTIBODY TO SOUTHERN ARMYWORM (SPODOPTERA ERIDANIA) MIDGUT NADPH-CYTOCHROME C REDUCTASE' see the whole document ---	1
X	MICROSC. ELECTRON BIOL. CEL., vol. 15, no. 2, 1991 pages 145-157, GUTIERREZ, L.S., ET AL. 'ANTIBODIES FROM CHAGAS PATIENTS SERUM BIND TO THE GUT EPITHELIAL CELL SURFACE OF TRIATOMA INFESTANS' see the whole document --- -/--	1

☒ Further documents are listed in the continuation of box C.☒ Patent family members are listed in annex.

* Special categories of cited documents:

- "A" document defining the general state of the art which is not considered to be of particular relevance
 "E" earlier document but published on or after the international filing date
 "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
 "O" document referring to an oral disclosure, use, exhibition or other means
 "P" document published prior to the international filing date but later than the priority date claimed

- "T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
 "X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
 "Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.
 "&" document member of the same patent family

Date of the actual completion of the international search

2 October 1995

Date of mailing of the international search report

17. 10. 95

Name and mailing address of the ISA

European Patent Office, P.B. 5818 Patentlaan 2
 NL - 2280 HV Rijswijk
 Td. (+31-70) 340-2040, Tx. 31 651 epo nl,
 Fax (+31-70) 340-3016

Authorized officer

Maddox, A

INTERNATIONAL SEARCH REPORT

Inter. Application No

PCT/IB 95/00497

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	J. EXP. ZOOL, vol. 251, no. 1, 1989 pages 108-112, AZUMA, M., ET AL. 'DISCRETE LOCALIZATION OF DISTINCT ALKALINE PHOSPHATASE ISOZYMES IN THE CELL SURFACE OF SILKWORM MIDGUT EPITHELIUM' see the whole document ---	1
A	TISSUE AND CELL, vol. 24, no. 5, 1992 pages 751-771, RYERSE, J.S., ET AL. 'PERITROPHIC MEMBRANE STRUCTURE AND FORMATION IN THE LARVA OF A MOTH, HELIOTHIS' see the whole document ---	1
A	FEBS LETTERS, vol. 307, no. 1, July 1992 AMSTERDAM NL, pages 71-75, HIATT, A., ET AL. 'MONOCLONAL ANTIBODY ENGINEERING IN PLANTS' see page 74, right column, paragraph 4 ---	1-56
A	NATURE, vol. 366, 2 December 1993 LONDON GB, pages 469-472, TAVLADORAKI, P., ET AL. 'TRANSGENIC PLANTS EXPRESSING A FUNCTIONAL SINGLE-CHAIN FV ANTIBODY ARE SPECIFICALLY PROTECTED FROM VIRUS ATTACK' see the whole document ---	1-56
A	NETH. J. PL. PATH. SUPPLEMENT 2, vol. 98, 1992 pages 183-191, SCHOTS, A., ET AL. 'PLANTIBODIES: A FLEXIBLE APPROACH TO DESIGN RESISTANCE AGAINST PATHOGENS' see the whole document ---	1-56
A	WO,A,91 17254 (UNIV CALIFORNIA) 14 November 1991 see the whole document ---	1-56
A	EP,A,0 438 312 (MERCK & CO INC) 24 July 1991 see figure 24 -----	8

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/IB 95/00497

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO-A-9117254	14-11-91	US-A- 5143905	01-09-92
		AU-A- 7865591	27-11-91
		CN-A- 1059760	25-03-92
		US-A- 5306628	26-04-94
EP-A-0438312	24-07-91	AU-B- 635996	08-04-93
		AU-A- 6984391	25-07-91
		CA-A- 2034574	20-07-91
		CA-A- 2034593	20-07-91
		EP-A- 0440351	07-08-91
		JP-A- 6054697	01-03-94
		JP-A- 6054698	01-03-94